Elucidation of the Interaction of Calmodulin with the IQ Motifs of IQGAP1*

Zhigang Li and David B. Sacks‡

From the Department of Pathology, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts 02115

Calmodulin regulates the function of numerous proteins by binding to short regions on the target molecule. IQ motifs, which are found in over 100 human proteins, appear in tandem repeats and bind calmodulin in the absence of Ca\(^{2+}\). One of these IQ-containing proteins, IQGAP1, interacts with several targets, including Cdc42, β-catenin, E-cadherin, and actin, in a calmodulin-regulated manner. To elucidate the molecular mechanism by which apocalmmodulin and Ca\(^{2+}\)/calmodulin differentially regulate IQGAP1, a series of constructs of IQGAP1 with selected point mutations of the four tandem IQ motifs were generated. Mutating the basic charged arginine residues in all four IQ motifs abrogated binding of IQGAP1 to apocalmmodulin, but had no effect on its interaction with Ca\(^{2+}\)/calmodulin. Analysis of IQGAP1 constructs with point mutations in single, double, or triple IQ motifs revealed that apocalmmodulin bound only to IQ3 and IQ4. By contrast to the arginine mutant constructs, mutation of selected hydrophobic residues in the IQ motifs produced an IQGAP1 protein incapable of binding either apocalmmodulin or Ca\(^{2+}\)/calmodulin. These results, which differ from the conventional model of Ca\(^{2+}\)-independent binding of calmodulin to IQ motifs, provide insight into the complexity of the molecular interactions between calmodulin and IQ motifs.

Calmodulin is a multifunctional signaling protein that elicits myriad effects in cells by modulating the function of target proteins (1–5). A diverse array of proteins are regulated by Ca\(^{2+}\)/calmodulin, ranging from the classic kinases (such as myosin light chain kinase and the Ca\(^{2+}\)/calmodulin kinase family) (1) to ion channels and anchrax (4, 5). The calmodulin targets have short (~14–26 amino acid residues) regions to which calmodulin binds. Although these domains exhibit little sequence conservation, many adopt an amphiphilic α-helical conformation (6). In addition to these Ca\(^{2+}\)-dependent targets, proteins that bind to calmodulin in the absence of Ca\(^{2+}\) were subsequently identified (7, 8). These targets contain a sequence called the IQ motif (9). Initially described in neuromodulin and unconventional myosins (9), examination of the Pfam data base reveals IQ motifs in over 100 human proteins. The IQ motif comprises 20–25 amino acids, with the core fitting the consensus IQXXXRGXXXR (where X is any amino acid) (9–11). IQ motifs frequently appear in tandem repeats that bind multiple calmodulin molecules with highest affinity in the absence of Ca\(^{2+}\) (12).

IQGAP1, a ubiquitous 190-kDa protein, contains several protein recognition motifs through which it interacts with targets (13, 14). Proteins that bind to and are regulated by IQGAP1 include E-cadherin (15, 16), β-catenin (15, 17), Cdc42 (18–21), and actin (19, 22, 23). In addition, calmodulin binds to the IQ region of IQGAP1 both in the presence and absence of Ca\(^{2+}\) (19, 24). In contrast to most IQ-containing proteins, IQGAP1 exhibits an affinity for Ca\(^{2+}\)/calmodulin ~2-fold higher than for apocalmmodulin (19, 24). Investigation of the functional sequelae of the interaction reveals that calmodulin modulates the binding of IQGAP1 to its other targets (16, 17, 19, 23, 24). Interestingly, calmodulin attenuates some of the IQGAP1-target interactions only in the presence of Ca\(^{2+}\) (19, 23). To elucidate the molecular mechanism by which Ca\(^{2+}\)/calmodulin and apocalmmodulin differentially regulate IQGAP1 function, we have generated a series of constructs of IQGAP1 with selected point mutations in each of the four tandem IQ motifs. Analysis of the binding of these constructs to calmodulin provides insight into our understanding of the mode of interaction between calmodulin and IQ motifs.

**EXPERIMENTAL PROCEDURES**

**IQGAP1 Plasmid Construction**—A Myc-tagged human IQGAP1 in pDNA2 vector (24) was used. Construction of IQGAP1C (residues 35–365) deleted), IQGAP1WW (residues 643–744 deleted), IQGAP1IQ (residues 899–905 deleted), and IQGAP1GRD (residues 1122–1324 deleted) mutants was described previously (16, 20). All deletion mutants migrated to the expected position on SDS-PAGE (see Fig. 1A). To perform site-directed mutagenesis, a Pcol linker was inserted into pBluescript KS at an SspI site to produce pBluescript-Pcol. An ~2-kilobase Pcol-ClaI fragment containing the IQ region of IQGAP1 was isolated from pDNA2-IQGAP1 and inserted into pBluescript-Pcol digested with Pcol and ClaI to produce pBluescript-IQ. Site-directed mutagenesis was performed with the QuikChange site-directed mutagenesis kit (Stratagene). After mutagenesis, the Pcol-ClaI fragment of pBluescript-IQ was re-inserted into pDNA2-IQGAP1 from which the wild type IQ region had been removed. The sequence of all constructs was confirmed by DNA sequencing. Plasmids were purified with a Qiaprep Spin Miniprep kit (Qiagen) according to the manufacturer’s instructions.

**Cell Culture and Transient Transfection**—COS and MCF-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) fetal bovine serum in a 37 °C humidified incubator with 5% CO\(_2\). Transient transfections of wild type or mutant IQGAP1 constructs were performed with FuGENE 6 (Roche Molecular Biochemicals) as instructed by the manufacturer. Briefly, cells were grown to 70–80% confluence in 100-mm dishes. Five micrograms of plasmid DNA was mixed with 15 μl of FuGENE 6 and added to the cells. After 24 to 48 h, cells were harvested, lysed, and processed as described below.

* This work was supported in part by a grant from the National Institutes of Health (to D. B. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Brigham and Women’s Hospital, Thorn 530, 75 Francis St., Boston, MA 02115. Tel.: 617-732-6627; Fax: 617-278-6921; E-mail: dsacks@rics.bwh.harvard.edu.
Binding Analysis—Mutant and wild type IQGAP1 cDNAs were subcloned into the pGEX-2T vector. Glutathione S-transferase (GST) fusion constructs of wild type and the indicated mutant constructs of IQGAP1 were expressed in Escherichia coli and isolated by glutathione-Sepharose essentially as described previously (19). All GST-IQGAP1 constructs were >90% pure (data not shown). Cells were lysed in 500 μl of buffer A (150 mM NaCl, 1% Triton X-100, and 50 mM Tris, pH 7.4) containing 1 mM CaCl2 or 1 mM EGTA. Equal amounts of protein lysate were precleared with glutathione-Sepharose beads for 1 h at 4°C. Lysates were then incubated with 500 ng of GST-IQGAP1 on glutathione-Sepharose beads for 3 h at 4°C. In all cases, GST alone was used as control. After sedimentation by centrifugation, samples were washed, resolved by SDS-PAGE, and transferred to polyvinylidene difluoride membrane. The resultant Western blots were probed with anti-Myc (16) antibody horseradish peroxidase-conjugated secondary antibody and developed with enhanced chemiluminescence (ECL). Where indicated, GST fusion proteins were incubated with 2 μg of calmodulin. Following SDS-PAGE, the gel was cut in half; the top portion (containing IQGAP1) was stained with Coomassie Blue and the bottom half was processed by blotting for calmodulin.

Binding to calmodulin was evaluated by calmodulin-Sepharose chromatography as described (24). Briefly, after preclearing with Sepharose beads for 1 h at 4°C, equal amounts of protein lysate were incubated with 40 μl of calmodulin-Sepharose (~20 μg of calmodulin) (or Sepharose without calmodulin as control) and incubated on a rotator at 4°C for 3 h. The calmodulin-Sepharose was washed five times in buffer A containing either 1 mM CaCl2 or 1 mM EGTA as appropriate and resuspended in SDS-PAGE sample buffer (20 mM Tris-HCl, pH 7.5, 2% (w/v) sodium dodecyl sulfate, 2% (v/v) β-mercaptoethanol, 0.01% (w/v) bromphenol blue, 0.25 mM sucrose, and 2 mM EGTA). Samples were heated at 100°C for 5 min and processed by immunoblotting as described above.

Miscellaneous—Densitometry of ECL signals was analyzed with UNSCAN-IT software (Silk Scientific Corp.). Statistical analysis was performed by Student’s t test, using Instat software (GraphPad Software, Inc.). Protein concentrations were measured with the DC protein assay (Bio-Rad).

RESULTS

Binding of Mutant IQGAP1 Constructs to Calmodulin—Initial analysis was performed with four deletion mutant IQGAP1 constructs. As previously demonstrated with endogenous IQGAP1, transfected wild type IQGAP1 bound readily to calmodulin in the absence of Ca2+ (Fig. 1A). Deletion of the CHD or the WW domains from IQGAP1 did not significantly change its affinity for calmodulin. By contrast, deletion of all four IQ motifs (IQGAP1ΔIQ) essentially eliminated binding (Fig. 1). Surprisingly, IQGAP1ΔGRD had significantly reduced binding to calmodulin, although the region deleted is more than 200 amino acids distal to the IQ domain. The reason is not known, but is presumably because of effects on the tertiary conformation of IQGAP1. The reduced binding is not caused by total disruption of IQGAP1 structure because both IQGAP1ΔIQ (17) and IQGAP1ΔGRD (data not shown) bind to β-catenin with an affinity similar to that of wild type IQGAP1. Interestingly, the conformational “cross-talk” between the IQ and GRD regions seems to be reciprocal. We previously observed that deletion of the IQ motif prevents Cdc42, which binds to the GRD and adjacent residues in the C-terminal half of the molecule (13), from co-immunoprecipitating with IQGAP1 (20). Binding was specific for calmodulin as wild type IQGAP1 did not bind to Sepharose alone (Fig. 1A). Essentially identical findings were observed in the presence of Ca2+ (data not shown).}

Selected Point Mutations of Basic Residues in the IQ Motifs of IQGAP1 Decrease Binding to Apocalmodulin, but Not to Ca2+/Calmodulin—Because deletion of all four IQ motifs abrogated the binding of IQGAP1 to calmodulin, point mutations were generated of individual residues in each IQ motif. A prior publication (26) indicated that substitution of Gln for the conserved Arg residues in the IQ motif of Ras-GRF prevented calmodulin binding. Moreover, binding of light chains to the IQ region of scallop myosin demonstrates that the conserved Arg residues in the IQ form important hydrogen bonds (27). For these reasons, we chose to mutate to Gln the Arg residues in the IQ motifs of IQGAP1. IQ1, IQ3, and IQ4 are complete IQ motifs, whereas IQ2 is incomplete as it lacks Arg at position 11 (11) (Fig. 2). Therefore, for IQ1, IQ3, and IQ4 we mutated the single Arg and proximal Gln (at position 2) were replaced by Gln and Ala, respectively (Fig. 2). These constructs were termed the IQR series, because the Arg residues were mutated.

Replacement of the charged Arg residues in all four IQ motifs (IQ1Δ2,3,4R) essentially eliminated binding to apocalmodulin (Fig. 3). To ascertain the relative contribution of individual IQ motifs to overall calmodulin binding, analysis was also performed using constructs with point mutations in single or multiple IQ motifs. Interestingly, replacement of the Arg residues in only IQ3 and IQ4 (IQ3,4ΔR) yielded a lack of binding essentially identical to that seen with mutation of all four IQ motifs (Fig. 3). By contrast, mutation of both IQ1 and IQ2 (IQ1,2ΔR) resulted in binding to calmodulin that was not significantly different from that of wild type IQGAP1. The constructs with mutation of IQ3 alone (IQ3ΔR) or IQ4 alone (IQ4ΔR) bound calmodulin with an affinity ~50% of that seen with wild type

1 The abbreviations used are: GST, glutathione S-transferase; GRD, Ras-GAP-related domain; GAP, GTPase activating protein; CHD, calponin homology domain; H, hydrophobic residue.
wild type IQGAP1 and represent the mean ± S.E. from three independent experimental determinations. *, significantly different from wild type (p < 0.001).

IQGAP1, whereas mutation of IQ1 alone (IQ1R) had no effect on binding (Fig. 3). These data imply that apocalmodulin binds only to IQ3 and IQ4, with approximately equal affinity for each of the two IQ motifs. This interpretation is supported by the observation that mutation of IQ1, IQ2, and IQ4 (IQ1,2,4R) yields a construct that binds calmodulin indistinguishably from IQ4R (Fig. 3), indicating that IQ1 and IQ2 do not bind apocalmodulin.

A markedly different effect was observed with Ca\(^{2+}\)/calmodulin. Mutation of Arg residues in one, two, three, or all four IQ motifs had no significant effect on the interaction of IQGAP1 with Ca\(^{2+}\)/calmodulin (Fig. 4). Ca\(^{2+}\)/calmodulin bound to IQ1,2,3,4R IQGAP1 with the same affinity as to wild type IQGAP1. The binding properties of IQ1,2,3,4R led us to rename this construct IQGAP1-apoCaM\(^{-}\).

Mutation of Hydrophobic Residues in the IQ Motifs of IQGAP1 Attenuates Calmodulin Binding—Because replacing the basic charged Arg residues in the IQ motifs with Gln failed to reduce binding to Ca\(^{2+}\)/calmodulin, an alternative strategy was adopted. Based on the mechanism by which calmodulin recognizes target peptides in the presence of Ca\(^{2+}\), hydrophobic amino acids in the IQ motifs were targeted (Fig. 5A). The prediction was that one of the lobes of calmodulin would interact with the H-1 residues, whereas the other calmodulin lobe would bind to the H-2 residues. Therefore, selected hydrophobic residues distal to the Gln (Q) of the IQ motif were mutated to Asp (Fig. 5A). All the H-1 and the distal H-2 (at position 14) residues were mutated. These point mutant constructs are termed the QH series (H for hydrophobic). Replacement of these hydrophobic residues (at positions 5, 8, and 14) in all four IQ motifs prevented binding to apocalmodulin (Fig. 5B).

Unexpectedly, the IQGAP1 construct with all four IQ motifs mutated as described (QH) still bound to Ca\(^{2+}\)/calmodulin, although the affinity was attenuated (Fig. 5C). Therefore, an additional point mutation was performed in the residue immediately proximal to the Gln (the “T” of the IQ). Leu-752 in IQ1 was changed to Glu, whereas the Ile in IQ2, IQ3, and IQ4 (Ile-782, Ile-812, and Ile-842, respectively) were converted to Asp (Fig. 5A). These constructs are termed the IQH series. Analysis of the IQ1,2,3,4H mutant with all four IQ motifs mutated revealed a complete absence of binding to apocalmodulin (Fig. 6B). Similarly, mutation of all four IQ motifs abrogated binding to Ca\(^{2+}\)/calmodulin. The IQ1,2,3,4H was re-
named IQGAP1-CaM⁻<sup>-</sup> because it does not bind calmodulin regardless of whether Ca<sup>2+</sup> is absent or present. As previously observed (24), more wild type IQGAP1 bound to Ca<sup>2+</sup>/calmodulin than to apocalmodulin (Fig. 6, compare lane 1 (WT) in calmodulin-Sepharose pull-downs in panels B and C).<br><br>**FIG. 5.** Mutation of selected hydrophobic residues in the IQ motifs of IQGAP1 modulates calmodulin binding. A, the four IQ motifs of IQGAP1 are aligned. The consensus sequence is at the bottom with hydrophobic (H) residues in bold. The residues depicted in bold and labeled with an asterisk in each IQ motif were mutated to Asp (D). B, COS cells were transiently transfected with wild type IQGAP1 (WT) or the point mutant constructs indicated. After 48 h, cells were lysed in buffer containing 1 mM EGTA and equal amounts of protein were incubated with calmodulin-Sepharose or Sepharose alone. Both unfractonated lysates (Lysate) and isolated complexes (CaM-Sepharose or Sepharose) were resolved by SDS-PAGE and Western blots were probed with anti-Myc antibody.<br><br>**FIG. 6.** Mutation of hydrophobic residues in the IQ motifs of IQGAP1 attenuates calmodulin binding. A, the four IQ motifs of IQGAP1 are aligned. The consensus sequence is at the bottom with hydrophobic (H) residues in bold. The residues depicted in bold and labeled with an asterisk in each IQ motif were mutated to Asp (D).<br><br>**Interaction of Calmodulin with IQ Motifs**<br><br>Moreover, consistent with the data obtained with calmodulin-Sepharose analysis, purified IQGAP1-apoCaM⁻<sup>-</sup> was unable to interact with calmodulin in the absence of Ca<sup>2+</sup>, but readily bound to Ca<sup>2+</sup>/calmodulin. The presence of the GST-IQGAP1...
wild type and mutant IQGAP1 in the presence or absence of 
Pure calmodulin was incubated with GST fusion proteins of 
were probed for calmodulin and developed by ECL.
A Complexes were pelleted by centrifugation, washed, and resolved by 
transferred to polyvinylidene difluoride and probed for calmodulin. Repre-
the gel was cut into two pieces; the top portion (containing IQGAP1) 
are not responsible for the altered calmodulin binding to the 
validate our findings and indicate that other proteins in the cell 
constructed in each sample was validated by probing blots for 
constructs in wild type and mutant IQGAP1 (data not shown).
Analysis was also performed in vitro with purified proteins.
Pure calmodulin was incubated with GST fusion proteins of 
bound no calmodulin, whereas IQGAP1-apoCaM bound 
Ca2+/calmodulin but not apocalmodulin (Fig. 7). These data 
also not be responsible for the altered calmodulin binding to the 
IQGAP1 mutants.

DISCUSSION

IQ motifs, first recognized as calmodulin-binding domains in 
neuromodulin (7, 8), have been identified in many proteins 
with a diverse array of functions (10). One of these is the 
scaffolding protein IQGAP1. In this paper, we generated two 
different series of point mutant constructs of the four tandem 
IQ motifs of IQGAP1, namely the IQR (Arg residues mutated) 
and IQH (hydrophobic residues mutated) series. Analysis of 
these constructs reveals that apocalmodulin binds to only IQ3 
and IQ4, whereas Ca2+/calmodulin binds to all four IQ motifs 
of IQGAP1 (note that the stoichiometry of CaM:IQGAP1 is 4:1 
(23)). We previously documented that Ca2+/calmodulin and 
apocalmodulin bind to fusion proteins of the IQ region of 
IQGAP1 (19). A low affinity binding site for Ca2+/calmodulin, 
but not apocalmodulin, was also identified in the calponin 
homology domain (CHD). The CHD appears to make a small 
contribution to total calmodulin binding as deletion of this 
region did not significantly reduce the binding of IQGAP1 to 
calmodulin. Moreover, essentially no binding of calmodulin 
was detected to IQGAP1 constructs with point mutations in the IQ 
motifs, despite the presence of an intact CHD.

X-ray and NMR structures of calmodulin bound to “classic” 
target peptides reveal that calmodulin is compact, with the 
from those anticipated from the model. For example, 
and IQH (hydrophobic residues mutated) series. Analysis of 
these constructs reveals that apocalmodulin binds to only IQ3 
and IQ4, whereas Ca2+/calmodulin binds to all four IQ motifs 
of IQGAP1 (note that the stoichiometry of CaM:IQGAP1 is 4:1 
(23)). We previously documented that Ca2+/calmodulin and 
apocalmodulin bind to fusion proteins of the IQ region of 
IQGAP1 (19). A low affinity binding site for Ca2+/calmodulin, 
but not apocalmodulin, was also identified in the calponin 
homology domain (CHD). The CHD appears to make a small 
contribution to total calmodulin binding as deletion of this 
region did not significantly reduce the binding of IQGAP1 to 
calmodulin. Moreover, essentially no binding of calmodulin 
was detected to IQGAP1 constructs with point mutations in the IQ 
motifs, despite the presence of an intact CHD.

Although IQ motifs were described 10 years ago (9), a de-
tailed structure of the calmodulin-IQ complex has not yet been 
published. A model of calmodulin bound to an IQ motif has 
been constructed, using the crystal structure of the regulatory 
domain of scallop myosin (11, 29). The highly conserved portion 
of the IQ motif (IQXXXR; the first residue may be Ile, Leu, or 
Val (10)) is the most critical region and determines both the 
conformation and positioning of the C-terminal lobe of calmo-
dulin (29). The second part of the IQ motif core (GXXXR) is not 
well conserved and has a minor role in fixing the position of the 
calmodulin N-terminal lobe. IQ motifs having both parts are 
term “complete,” whereas those lacking the second part are 
term “incomplete.” This distinction is important because 
binding of calmodulin to complete IQ motifs does not require 
Ca2+ (29). The C-terminal lobe of calmodulin is expected to be 
semi-open and the N-terminal lobe closed when bound to a 
complete IQ motif. By contrast, when bound to an incomplete 
IQ motif, the N-terminal lobe would adopt an open confor-
mation provided that Ca2+ is present (29). It is thought that 
apocalmodulin and Ca2+/calmodulin are likely to bind different 
sites in the IQ motif (11).

The results presented here complement the model. Some of 
our data support the scheme, whereas other observations differ 
from those anticipated from the model. For example, consistent 
with the predictions of Houdusse and Cohen (11), both hydro-
phobic and electrostatic interactions appear to contribute to the 
-binding of apocalmodulin and both seem necessary; disruption 
of either mode of association by mutation of critical residues 
reduces binding. The expectation from the model was that 
apocalmodulin would bind all the complete IQ motifs. However, 
our data revealed that apocalmodulin did not bind to IQ1, 
although it fulfills the criteria of a complete IQ motif. As 
indicated by our results, caution should be exercised in extrap-
olating data obtained with model IQ peptides because these do 
not always mimic the behavior of the intact protein. For ex-
ample, neuromodulin and neurogranin bind calmodulin only in 
the absence of Ca2+, but isolated peptides of the calmodulin-
binding domains bind Ca2+/calmodulin (30–32). Similarly, the 
interaction of calmodulin with the complete catalytic domain of 
edema factor of adenylly cyclase was different from its inter-
action with the peptide of the calmodulin-binding domain; the 
peptide induced a conformation of calmodulin opposite to that 
caused by the whole catalytic domain (5). These observations 
emphasize the importance of studies with intact proteins as 
conducted here.

It is generally believed that calmodulin targets that contain 
IQ motifs have a higher affinity for the Ca2+-free form of 
calmodulin (8, 12, 31, 33, 34). Moreover, for some proteins such 
as brush border myosin I (35), Ca2+ induces the dissociation of
bound calmodulin. Our mutagenesis data reveal that the interaction between calmodulin and IQ motifs is substantially more complex than current thinking and selected IQ motifs may have higher affinity for Ca\(^{2+}\)/calmodulin. This observation is supported by the reports from both our laboratory and others that the IQ-containing proteins IRS-1 (36) and NicaA myosin (37) bind Ca\(^{2+}\)/calmodulin with higher affinity than apocalmodulin. The results presented here extend previous findings, indicating that the binding of Ca\(^{2+}\)/calmodulin to IQ motifs exhibits several features different from those of apocalmodulin.

First, Ca\(^{2+}\)/calmodulin binds to all four IQ motifs of IQGAP1, whereas apocalmodulin does not interact appreciably with the first two (IQ1 or IQ2) IQ motifs. These findings provide the molecular mechanism for our initial observation that 2-fold more IQGAP1 bound calmodulin in the presence of Ca\(^{2+}\) (24). Second, electrostatic interactions appear less important for Ca\(^{2+}\)/calmodulin binding than hydrophobic interactions. Substitution of the polar but uncharged Gln for critical basic Arg residues in all four IQ motifs did not attenuate the interaction with calmodulin, but exhibits several features different from those of apocalmodulin.

Further, conservation and Ca\(^{2+}\)/calmodulin binding to Ca\(^{2+}\)/calmodulin binding than hydrophobic interactions. Substitution of the polar but uncharged Gln for critical basic Arg residues in all four IQ motifs did not attenuate the interaction with calmodulin, but exhibits several features different from those of apocalmodulin. The I of the IQ motif appears to be essential for Ca\(^{2+}\)/calmodulin binding, whereas IQ1 and IQ2 bind only Ca\(^{2+}\)/calmodulin. The I of the IQ motif appears to be essential for Ca\(^{2+}\)/calmodulin binding, whereas IQ1 and IQ2 bind only Ca\(^{2+}\)/calmodulin. The I of the IQ motif appears to be essential for Ca\(^{2+}\)/calmodulin binding, whereas IQ1 and IQ2 bind only Ca\(^{2+}\)/calmodulin.

The functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. For the unconventional myosins, IQ motifs are thought to influence the chemomechanical properties of the myosins (10). Binding of calmodulin has a substantial effect on IQGAP1, modulating its interaction with one another or to other regions of IQGAP1. The observation that IQGAP1apoCaM\(^{-}\) and IQ1,2,3,4 IQGAP1 (which have 7 and 10 substitutions, respectively) bind to Ca\(^{2+}\)/calmodulin with the same affinity as to wild type IQGAP1 renders this possibility less likely. It remains possible, however, that the mutant IQGAP1 could bind to calmodulin by an interaction different from that in the native protein. This premise can be eliminated only by solving the structures of wild type and mutant IQGAP1.

The functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. For the unconventional myosins, IQ motifs are thought to influence the chemomechanical properties of the myosins (10). Binding of calmodulin has a substantial effect on IQGAP1, modulating its interaction with one another or to other regions of IQGAP1. The observation that IQGAP1apoCaM\(^{-}\) and IQ1,2,3,4 IQGAP1 (which have 7 and 10 substitutions, respectively) bind to Ca\(^{2+}\)/calmodulin with the same affinity as to wild type IQGAP1 renders this possibility less likely. It remains possible, however, that the mutant IQGAP1 could bind to calmodulin by an interaction different from that in the native protein. This premise can be eliminated only by solving the structures of wild type and mutant IQGAP1.

The functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. For the unconventional myosins, IQ motifs are thought to influence the chemomechanical properties of the myosins (10). Binding of calmodulin has a substantial effect on IQGAP1, modulating its interaction with one another or to other regions of IQGAP1. The observation that IQGAP1apoCaM\(^{-}\) and IQ1,2,3,4 IQGAP1 (which have 7 and 10 substitutions, respectively) bind to Ca\(^{2+}\)/calmodulin with the same affinity as to wild type IQGAP1 renders this possibility less likely. It remains possible, however, that the mutant IQGAP1 could bind to calmodulin by an interaction different from that in the native protein. This premise can be eliminated only by solving the structures of wild type and mutant IQGAP1.

The functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. For the unconventional myosins, IQ motifs are thought to influence the chemomechanical properties of the myosins (10). Binding of calmodulin has a substantial effect on IQGAP1, modulating its interaction with one another or to other regions of IQGAP1. The observation that IQGAP1apoCaM\(^{-}\) and IQ1,2,3,4 IQGAP1 (which have 7 and 10 substitutions, respectively) bind to Ca\(^{2+}\)/calmodulin with the same affinity as to wild type IQGAP1 renders this possibility less likely. It remains possible, however, that the mutant IQGAP1 could bind to calmodulin by an interaction different from that in the native protein. This premise can be eliminated only by solving the structures of wild type and mutant IQGAP1.

The functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. For the unconventional myosins, IQ motifs are thought to influence the chemomechanical properties of the myosins (10). Binding of calmodulin has a substantial effect on IQGAP1, modulating its interaction with one another or to other regions of IQGAP1. The observation that IQGAP1apoCaM\(^{-}\) and IQ1,2,3,4 IQGAP1 (which have 7 and 10 substitutions, respectively) bind to Ca\(^{2+}\)/calmodulin with the same affinity as to wild type IQGAP1 renders this possibility less likely. It remains possible, however, that the mutant IQGAP1 could bind to calmodulin by an interaction different from that in the native protein. This premise can be eliminated only by solving the structures of wild type and mutant IQGAP1.

The functional sequelae of calmodulin binding to IQ motifs remain incompletely understood. For the unconventional myosins, IQ motifs are thought to influence the chemomechanical properties of the myosins (10). Binding of calmodulin has a substantial effect on IQGAP1, modulating its interaction with one another or to other regions of IQGAP1. The observation that IQGAP1apoCaM\(^{-}\) and IQ1,2,3,4 IQGAP1 (which have 7 and 10 substitutions, respectively) bind to Ca\(^{2+}\)/calmodulin with the same affinity as to wild type IQGAP1 renders this possibility less likely. It remains possible, however, that the mutant IQGAP1 could bind to calmodulin by an interaction different from that in the native protein. This premise can be eliminated only by solving the structures of wild type and mutant IQGAP1.