Biochemical analysis of the interactions of IQGAP1 C-terminal domain with CDC42.


Published in:
World journal of biological chemistry

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
© 2012 Baishideng. This is an open access Creative Commons Attribution-NonCommercial License (https://creativecommons.org/licenses/by-nc/4.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen’s institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person’s rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>REVIEW</td>
<td>41</td>
<td>Transforming growth factor-β and smooth muscle differentiation</td>
<td>Guo X, Chen SY</td>
</tr>
<tr>
<td>BRIEF ARTICLES</td>
<td>53</td>
<td>Biochemical analysis of the interactions of IQGAP1 C-terminal domain with CDC42</td>
<td>Elliott SF, Allen G, Timson DJ</td>
</tr>
</tbody>
</table>
Contents

World Journal of Biological Chemistry
Volume 3 Number 3 March 26, 2012

ACKNOWLEDGMENTS

Acknowledgments to reviewers of World Journal of Biological Chemistry

APPENDIX

Meetings

Instructions to authors

ABOUT COVER

Editorial Board Member of World Journal of Biological Chemistry, Hui-Ling Chiang, PhD, Professor, Department of Cellular and Molecular Physiology, Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, United States

AIM AND SCOPE

World Journal of Biological Chemistry (World J Biol Chem, WJBC, online ISSN 1949-8454, DOI: 10.4331), is a monthly, open-access, peer-reviewed journal supported by an editorial board of 529 experts in biochemistry and molecular biology from 40 countries.

The major task of WJBC is to rapidly report the most recent developments in the research by the close collaboration of biologists and chemists in the area of biochemistry and molecular biology, including: general biochemistry, pathobiochemistry, molecular and cellular biology, molecular medicine, experimental methodologies and the diagnosis, therapy, and monitoring of human disease.

FLYLEAF

Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: Jian-Xia Cheng
Responsible Electronic Editor: Dan-Ni Zhang
Proofing Editor-in-Chief: Lian-Sheng Ma

NAME OF JOURNAL
World Journal of Biological Chemistry

ISSN
ISSN 1949-8454 (online)

LAUNCH DATE
February 26, 2010

FREQUENCY
Monthly

EDITING
Editorial Board of World Journal of Biological Chemistry, Room 903, Building D, Ocean International Center, No. 62 Dongshihua Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjbc@wjgnet.com
http://www.wjgnet.com

EDITOR-IN-CHIEF
Yin-Yuan Mo, PhD, Associate Professor, Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, IL 62702, United States

EDITION OFFICE
Jian-Xia Cheng, Director
World Journal of Biological Chemistry
Room 903, Building D, Ocean International Center, No. 62 Dongshihua Zhonglu, Chaoyang District, Beijing 100025, China

Telephone: +86-10-85381892
Fax: +86-10-85381893
E-mail: wjbc@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited, Room 1701, 17/F, Henan Building, No.90 Jaffe Road, Wanchai, Hong Kong, China

Telephone: +852-31158812
Fax: +852-39042046
E-mail: bjpc@baishideng.com
http://www.wjgnet.com

PUBLICATION DATE
March 26, 2012

COPYRIGHT
© 2012 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1949-8454/g_info_20100316155305.htm

ONLINE SUBMISSION
http://www.wjgnet.com/1949-8454office/
Biochemical analysis of the interactions of IQGAP1 C-terminal domain with CDC42

Sarah F Elliott, George Allen, David J Timson

Sarah F Elliott, George Allen, David J Timson, School of Biological Sciences, Queen’s University Belfast, Medical Biology Centre, Belfast, BT9 7BL, United Kingdom

Author contributions: Elliott SF carried out all protein expression and purification and the crosslinking experiments; Elliott SF and Allen G jointly performed the surface plasmon resonance measurements; Elliott SF, Allen G and Timson DJ analysed these data; Timson DJ carried out the molecular modelling work, was responsible for the overall design of the study, obtained research grants to support the work and wrote the manuscript.

Supported by The Biotechnology and Biological Sciences Research Council (BBSRC), United Kingdom, No. BB/D000394/1; Action Cancer, Northern Ireland, United Kingdom, No. PG2 2005

Correspondence to: Dr. David J Timson, School of Biological Sciences, Queen’s University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, BT9 7BL, United Kingdom. d.timson@qub.ac.uk

Telephone: +44-28-9097-5875 Fax: +44-28-9097-5877

Received: December 7, 2011 Revised: January 31, 2012 Accepted: February 7, 2012 Published online: March 26, 2012

Abstract

AIM: To understand the interaction of human IQGAP1 and CDC42, especially the effects of phosphorylation and a cancer-associated mutation.

METHODS: Recombinant CDC42 and a novel C-terminal fragment of IQGAP1 were expressed in, and purified from, Escherichia coli. Site directed mutagenesis was used to create coding sequences for three phosphomimicking variants (S1441E, S1443D and S1441E/S1443D) and to recapitulate a cancer-associated mutation (M1231I). These variant proteins were also expressed and purified. Protein-protein crosslinking using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide was used to investigate interactions between the C-terminal fragment and CDC42. These interactions were quantified using surface plasmon resonance measurements.

Molecular modelling was employed to make predictions about changes to the structure and flexibility of the protein which occur in the cancer-associated variant.

RESULTS: The novel, C-terminal region of human IQGAP1 (residues 877-1558) is soluble following expression and purification. It is also capable of binding to CDC42, as judged by crosslinking experiments. Interaction appears to be strongest in the presence of added GTP. The three phosphomimicking mutants had different affinities for CDC42. S1441E had an approximately 200-fold reduction in affinity compared to wild type. This was caused largely by a dramatic reduction in the association rate constant. In contrast, both S1443D and the double variant S1441E/S1443D had similar affinities to the wild type. The cancer-associated variant, M1231I, also had a similar affinity to wild type. However, in the case of this variant, both the association and dissociation rate constants were reduced approximately 10-fold. Molecular modelling of the M1231I variant, based on the published crystal structure of part of the C-terminal region, revealed no gross structural changes compared to wild type (root mean square deviation of 0.564 Å over 5556 equivalent atoms). However, predictions of the flexibility of the polypeptide backbone suggested that some regions of the variant protein had greatly increased rigidity compared to wild type. One such region is a loop linking the proposed CDC42 binding site with the helix containing the altered residue. It is suggested that this increase in rigidity is responsible for the observed changes in association and dissociation rate constants.

CONCLUSION: The consequences of introducing negative charge at Ser-1441 or Ser-1443 in IQGAP1 are different. The cancer-associated variant M1231I exerts its effects partly by rigidifying the protein.

© 2012 Baishideng. All rights reserved.

Key words: CDC42; Cytoskeleton; Protein phosphorylation;
Cancer-associated mutation; Protein-protein interaction

Peer reviewers: Guillermo Montoya, Dr., Department of Structural Biology and Biocomputing, Spanish National Cancer Research Centre (CNIO), Spanish National Cancer Research Centre (CNIO) Melchor Fdez. Almagro 3, 28029 Madrid, Spain; Yi Wang, Associate Professor, Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, BCM125, Houston, TX 77030, United States; Chin-Chuan Wei, Professor, Department of Chemistry, Southern Illinois University Edwardsville, PO Box 1652, Edwardsville, IL 62026, United States; Yu Jiang, Associate Professor, Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15261, United States


INTRODUCTION

The IQGAP family of proteins function at the interface between cellular signalling and the cytoskeleton\(^1\)-\(^3\). They receive information from a variety of signalling molecules, including kinases, small GTPases, growth factor receptors and calcium sensors\(^4\)-\(^21\). This information is relayed directly to the actin cytoskeleton through interaction with filamentous actin (F-actin) which promotes filament bundling and caps the “barbed ends” of the filaments\(^22\)-\(^25\).

There are also indirect influences on the actin cytoskeleton mediated through the Wiskott-Aldrich Syndrome Protein (WASP) family\(^24\),\(^26\) and with microtubules mediated via cytoplasmic linker protein 170 (CLIP-170) and adenomatous polyposis coli (APC) protein\(^27\),\(^28\). The IQGAP proteins are named after two key regions within them—the calmodulin binding IQ-motifs and GTPase activating protein (GAP) related domain (GRD). Although the GRD does bind the small GTPases CDC42 and Rac1\(^29\), it does not function as the GTPase activator. Indeed, the available evidence suggests that it inhibits the catalytic activity of small GTPases\(^30\). This is consistent with sequence data and structural predictions. GAPs function by inserting an “arginine finger” into the active site of small GTPases which acts as a proton donor in the enzymatic mechanism of GTP hydrolysis\(^30\)-\(^31\). IQGAPs lack this arginine residue and are thus not expected to be able to enhance the rate of GTP hydrolysis.

Humans have three IQGAP isoforms, IQGAP1, IQGAP2 and IQGAP3 with the first of these being the best characterised\(^32\). Like family members from other species they share a common domain organisation in which the actin-binding calponin homology domain (CHD) is at the N-terminus of the protein, the IQ-motifs are approximately in the middle of the primary sequence and the GRD is towards the C-terminus (Figure 1). There is no complete, three-dimensional structure of an IQGAP available, but it is assumed that the various domains fold in such a way to enable communication between them. It is also anticipated that there is considerable capacity for conformational change in the molecule in order to receive, integrate, interpret and output signals. The structures of some isolated domains have been determined. The structure of the CHD from human IQGAP1 has been solved by NMR spectroscopy and an x-ray structure of part of the GRD is also available\(^33\),\(^34\). Molecular modelling has predicted largely \(\alpha\)-helical structures for the IQ-motifs\(^35\)-\(^39\).

In vitro biochemical studies on IQGAPs have tended to rely on fragments of the protein which are amenable to recombinant expression and purification in bacterial systems. CDC42 and Rac1 interaction with the GRD is promoted by the presence of GTP\(^40\). Phosphorylation of human IQGAP1 at Ser-1443, however, promotes interaction with CDC42 in the absence of nucleotide\(^36\). This phosphorylation, along with one at Ser-1441, promotes outgrowth of neurites\(^37\).

Given the protein’s involvement in the transduction of information from signalling pathways to the cytoskeleton, it is not surprising that it has been implicated in various types of cancer\(^38\),\(^39\). However, only one cancer-associated mutation in the coding sequence of the \(Iqgap1\) gene has been identified; this mutation results in the amino acid change M1231I\(^40\). It is not clear how this change affects the function of IQGAP1, although it does lie in the GRD prompting the hypothesis that it interferes with GTPase binding. However, this has not been tested experimentally.

Here, we identified a novel, biochemically amenable fragment from the C-terminal region of human IQGAP1 and confirmed that it is active, as judged by its ability to bind CDC42 in a crosslinking experiment. We then describe a detailed, quantitative investigation into the affinity of this interaction in the absence of added GTP. To probe the molecular consequences of phosphorylation in this region we used “phosphomimicking” variants in which serine residues are replaced with negatively charged ones. We also recapitulated the cancer-associated variant M1231I in order to investigate its binding properties and carried out molecular modelling studies to provide further understanding of the consequences of this alteration.

MATERIALS AND METHODS

Expression and purification of wild type and variant human IQGAP C-terminal region

The sequence encoding amino acids 877-1558 in human IQGAP1 was amplified by polymerase chain reaction
Expression and purification of human CDC42

The complete coding sequence of human CDC42 was amplified by PCR using IMAGE clone 3626647[44] as a template and inserted into pET-46 Ek/LIC. The DNA sequence of the insert was verified. The expression and purification of the protein was carried out using the same protocol as for IQGAP1-CTD.

Crosslinking of CDC42 and IQGAP

GTP bound CDC42 was prepared by incubating a mixture of 6 μmol/L CDC42, 0.9 mmol/mol GTP and 0.9 magnesium chloride on ice for 30 min. Nucleotide-depleted (ND) CDC42 was prepared by incubating 6 μmol/L CDC42 with 5 mmol/L EDTA on ice for 30 min. Protein-protein crosslinking was carried out using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Methods were based on those previously used for the detection of interaction between the atrial myosin essential light chain and F-actin[45,46]. Untreated, GTP-loaded or ND CDC42 (3 μmol/L) was mixed with IQGAP(DR6) (3 μmol/L) and incubated for 30 min at 22 °C. EDC was then added to a final concentration of 0.6 mmol/L and the incubation continued for a further 60 min. Products were analysed by SDS-PAGE.

Surface plasmon resonance

Surface plasmon resonance was measured using a BIACore 3000 instrument (BIACore, Uppsala, Sweden). Prior to analysis all proteins were dialysed into HBS Buffer (BIACore; 10 mmol/L Hepes, pH 7.4, 150 mmol/L NaCl). CDC42 was immobilised onto a CM5 sensor chip (BIACore) using N-hydroxysuccinimide (NHS)/EDC chemistry. The surface was activated with a mixture of 100 mmol/L NHS and 400 mmol/L EDC for 30 min. CDC42 (25 μmol/L) was then flowed over the surface for two 7 min periods and the surface was then blocked and deactivated with 1 M ethanolamine for 30 min. Immobilisation of CDC42 resulted in a change in the response units (RU) of approximately 1400 RU.

Binding was measured by flowing 0.5 μmol/L to 2.5 μmol/L IQGAP(DR6) over the surface for 300 s (association phase) followed by buffer for 300 s (dissociation phase). In between binding measurements, the surface was regenerated by the injection of sodium hydroxide (5 mmol/L for 220 s). For each binding measurements controls were carried out in parallel in which the protein was flowed over a cell which had been activated with NHS/EDC and blocked with ethanolamine. To determine the response due to interaction between IQGAP(DR6) and CDC42, the readings for the controls were subtracted from the experimental ones. The association and dissociation rate constants (kₐ and k₅ respectively) and the dissociation equilibrium constant (Kₒ) were determined by non-linear curve fitting of the data using BIAevaluation software.

Molecular modelling

The structure of human IQGAP1, residues 962-1345 (PDB 3FYA)[44] was taken as a starting point for molecular modelling studies. This structure file describes one, unbroken polypeptide chain. The selenomethionine residues in this structure were altered to methionine using PyMol (www. pymol.org) and the resulting structure energy minimised using YASARA[47]. Residue 1231 in this minimised structure was altered to isoleucine, and the mutated structure

(PCR) using the Kazusa cDNA clone KIAA0051[43] as a template. The sequence was inserted into the pET-46 Ek/LIC (Merck, Nottingham, United Kingdom) by ligation independent cloning according to the manufacturer’s instructions. Insertion into this vector introduces a coding sequence encoding the amino acids MAHHHHHHVDDDDK at the 5'-end of the coding sequence. The complete coding sequence was verified (MWG Biotech, Ebersburg, Germany). The plasmid was transformed into competent Escherichia coli (E. coli) HMS174(DE3). Colonies resulting from these transformations were picked and grown, shaking at 37 °C, overnight in 5 mL of Luria-Bertani medium supplemented with 100 μg/mL of ampicillin. This overnight culture was diluted in to 1 L of Luria- Bertani medium supplemented with 100 μg/mL of ampicillin and grown, shaking at 37 °C until the cell density, as estimated by the A₆₀₀nm reached 0.6 to 1.0 (typically 3-4 h). The culture was induced by the addition of 1 mmol/L IPTG and grown, shaking at 37 °C until the cell density was approximately 10⁶ cells/mL. The cell suspension was then centrifuged (20 000 g for 15 min), resuspended in 20 mL of buffer R [50 mmol/L Hepes-OH, pH 7.5, 150 mmol/L sodium chloride, 10%(v/v) glycerol] and stored, frozen at -80 °C until required.

These cell suspensions were thawed and then disrupted by sonication (three 30 s pulses of 100 W, with 30-60 s gaps in between to allow cooling of the cells). Cell debris was removed by centrifugation (20 000 g for 15 min) and the supernatant applied to a 1 mL nickel-agarose column (His-Select, Sigma, Poole, United Kingdom) which had been previously equilibrated in buffer A [50 mmol/L Hepes-OH, pH 7.5, 500 mmol/L sodium chloride, 10%(v/v) glycerol]. The cell extract was allowed to pass through the column by gravity flow and the column was washed with 20 mL of buffer A. Protein was eluted with three 2 mL washes of buffer B (buffer A supplemented with 250 mmol/L imidazol). Protein containing fractions were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and dialysed overnight at 4 °C against buffer D (buffer R supplemented with 2 mmol/L dithiothreitol). The protein concentration was determined by the method of Bradford[45], using bovine serum albumin as a standard. Aliquots (50-100 μL) of the protein were stored frozen at -80 °C.

Mutations were introduced in to the coding sequence using the “QuikChange” protocol[43] and verified by DNA sequencing. Each variant protein was expressed in E. coli using bovine serum albumin as a standard. Aliquots (50-100 μL) of the protein were stored frozen at -80 °C.

Expression and purification of human CDC42

The complete coding sequence of human CDC42 was amplified by PCR using IMAGE clone 3626647[44] as a template and inserted into pET-46 Ek/LIC. The DNA sequence of the insert was verified. The expression and purification of the protein was carried out using the same protocol as for IQGAP1-CTD.
Identification of a biochemically tractable C-terminal fragment of human IQGAP1

Previous reports demonstrated that a fragment beginning at residue 864 and continuing through to the C-terminus of the protein (residue 1657) can be expressed in, and purified from, *E. coli*, albeit at relatively low levels\[^{18,19}\]. We noted that the structure of the Ras-GAP C-terminal domain (RGD), a 112 amino acid residue region at the extreme C-terminus of the protein has been deposited in the Protein Data Bank (PDB ID: 1X0H). From this we reasoned that there must be a domain boundary in the region of residue 1545. Therefore, a region beginning at residue 877 and finishing at 1558 was expressed. This fragment, which we named IQGAP1(DR6), can be purified with good yield, typically 1-2 mg per litre of *E. coli* culture (Figure 2A). Similar purities and yields were achieved with the various variant proteins also described in this work (data not shown). Full length, recombinant, human CDC42 could also be purified in good yield (Figure 2B).

**RESULTS**

**Identification of a biochemically tractable C-terminal fragment of human IQGAP1**

Recombinant, human CDC42 was shown to interact with the C-terminal domain fragment. The two proteins could be cross-linked using the reagent EDC which is specific to carboxylate and amino groups (Figure 3). This demonstrates that the recombinant C-terminal fragment is likely to be folded and is functional. The amount of crosslinked product was greatest in the presence of GTP (Figure 3).

**Effects of phosphomimicking mutations**

To investigate the effects of phosphorylation at serines 1441 and 1443, the phosphomimic variants S1441E, S1443D and S1441E/S1443D were constructed. These amino acid changes insert negative charges into the structure at the sites which can be phosphorylated. Similar mutants have been shown to recapitulate the effects of phosphorylation in an in vitro cell model\[^{37}\]. Since it has been hypothesised that phosphorylation increases the affinity for CDC42 in the absence of GTP\[^{8}\], this interaction was investigated by surface plasmon resonance. Interaction between the wild type and immobilised CDC42 could be detected by surface plasmon resonance in the absence of added GTP (Figure 4). Fitting of these data resulted in rate constants for the association and dissociation phases of the reactions (\(k_a\) and \(k_d\)) and, consequently, a value for the dissociation constant (\(K_d\)) (Table 1). It was noted that these fits were not perfect with some non-random residuals (not shown). This may indicate that there is heterogeneity in the preparations and/or that the binding event is more complex. However, for the purposes of comparison, the simple bimolecular interaction model was used...
All three phosphomimic variants also bound to CDC42 in the absence of additional nucleotide. However, in the case of S1441E, the affinity was reduced by two orders of magnitude. This arises mainly because of a reduction in the association rate constant. It should be noted that this reduced value \(12 \text{ l.mol}^{-1} \text{s}^{-1}\) is very low and, therefore, may be subject to greater error than the other values. Interestingly, the double mutant (S1441E/S1443D) binds with a similar affinity to the wild type (Table 1).

### Effects of the cancer-associated mutation, M1231I

The ability of the disease-associated variant to interact with CDC42 was tested by surface plasmon resonance. These experiments suggest that it is able to do so with similar affinity to the wild type protein. However, both the association and dissociation rate constants are reduced compared to wild type (Table 1).

To help understand the biochemistry of the M1231I variant protein, a molecular model was constructed based on the crystal structure of the GRD. This suggested that the overall fold is not greatly changed by the substitution of this methionine for isoleucine (rmsd between the wild type and variant protein 0.564 Å over 5556 equivalent atoms). The residue lies towards the surface of the protein, away from the predicted GTPase binding site. In addition to gross structural changes, the functions of proteins can be affected by the flexibility of the molecule. Computationally estimation of the backbone flexibility of the molecule suggested that the M1231I variation results in changes in flexibility at a number of sites within the protein (Figure 5A). The site with the greatest loss of flexibility is a loop (Ser-1212 to Leu-1217) which links the \(\alpha\)-helix containing residue 1231 with residues predicted to play a key role in the CDC42 binding site (Tyr-1192 to Arg-1194; Figure 5B). This loss of flexibility may affect the dynamics of small GTPase interaction.

### DISCUSSION

These experiments establish a new fragment from the C-terminal region of human IQGAP1 which is amenable to biochemical analysis. The fragment interacts with CDC42, and the strength of interaction is increased in throughout.

All three phosphomimic variants also bound to CDC42 in the absence of additional nucleotide. However, in the case of S1441E, the affinity was reduced by two orders of magnitude. This arises mainly because of a reduction in the association rate constant. It should be noted that this reduced value \(12 \text{ l.mol}^{-1} \text{s}^{-1}\) is very low and, therefore, may be subject to greater error than the other values. Interestingly, the double mutant (S1441E/S1443D) binds with a similar affinity to the wild type (Table 1).

**Table 1** Binding parameters for the interaction of IQGAP1(DR6) and CDC42

<table>
<thead>
<tr>
<th>IQGAP1(DR6) variant</th>
<th>(k_a/\text{l.mol}^{-1} \text{s}^{-1})</th>
<th>(k_d/\text{s}^{-1})</th>
<th>(K_D/\mu\text{mol/L})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>(4900 \pm 100) (6.5 \pm 0.5 \times 10^4)</td>
<td>(1.3 \pm 0.13)</td>
<td></td>
</tr>
<tr>
<td>S1441E</td>
<td>(12 \pm 1) (2.7 \pm 0.03 \times 10^4)</td>
<td>(220 \pm 21)</td>
<td></td>
</tr>
<tr>
<td>S1443D</td>
<td>(5800 \pm 100) (4.7 \pm 0.2 \times 10^4)</td>
<td>(0.81 \pm 0.048)</td>
<td></td>
</tr>
<tr>
<td>S1441E/S1443D</td>
<td>(3600 \pm 100) (4.0 \pm 0.2 \times 10^4)</td>
<td>(1.1 \pm 0.086)</td>
<td></td>
</tr>
<tr>
<td>M1231I</td>
<td>(1800 \pm 100) (1.7 \pm 0.2 \times 10^4)</td>
<td>(0.90 \pm 0.16)</td>
<td></td>
</tr>
</tbody>
</table>

These were determined by surface plasmon resonance. The values are reported ± their standard errors as determined by the BLAevaluation fitting programme (see Materials and Methods).
the presence of GTP. Interestingly, previous work has demonstrated, using isothermal titration calorimetry, an interaction between a C-terminal fragment of IQGAP (residues 962-1345) and GTP-loaded CDC42, but not with CDC42 purified in the absence of added nucleotides (assumed to be GDP-loaded)\cite{1}. This may indicate that the additional residues present in the IQGAP1(DR6) fragment are important in CDC42 interaction in the absence of GTP. The phosphomimicking variants suggest that phosphorylation of the two serine residues has different effects. While the S1443D variant has slightly increased affinity for CDC42, the affinity of S1441E is decreased and introduction of a negative charge at both sites restores the affinity to essentially wild type levels. This suggests that there may be crosstalk between the two serines within the C-terminal domain. The results from the cancer-associated variant emphasise the impor-

\section*{ACKNOWLEDGMENTS}

We thank Elaine Hamilton and Kai Chi Chan for their assistance in the early stages of this project.

\section*{REFERENCES}

1 Brown MD, Sacks DB. IQGAP1 in cellular signaling: bridging the GAP. Trends Cell Biol 2006; 16: 242-249
2 Briggs MW, Sacks DB. IQGAP proteins are integral components of cytoskeletal regulation. EMBO Rep 2003; 4: 571-574
3 Briggs MW, Sacks DB. IQGAP1 as signal integrator: Ca2+, calmodulin, Cdc42 and the cytoskeleton. FEBS Lett 2003; 542: 7-11
4 Hart MJ, Callow MG, Souza B, Polakis P, IQGAP1, a calmodulin-binding protein with a rasGAP-related domain, is a potential effector for cdc42Hs. EMBO J 1996; 15: 2997-3005
5 Weissbach L, Settlemidor J, Kalady MF, Sijanders AJ, Murthy AE, Yan YX, Bernards A. Identification of a human rasGAP-


9  Ren JG, Li Z, Sacks DB. IQGAP1 modulates activation of B-Raf. *Proc Natl Acad Sci USA* 2007; 104: 10465-10469


11 Li Z, Sacks DB. Elucidation of the interaction of calmodulin with the IQ motifs of IQGAP1. *J Biol Chem* 2003; 278: 4347-4352


13 Swart-Mataraza JM, Li Z, Sacks DB. IQGAP1 is a component of Cdc42 signaling to the cytoskeleton. *J Biol Chem* 2002; 277: 24753-24763


23 Osman MA, Cerione RA. Igglp1, a yeast homologue of the mammalian IQGAPs, mediates cdc42p effects on the actin cytoskeleton. *J Cell Biol* 1998; 142: 443-455


33 Uemoto M, Nishiuda N, Ogino S, Shimada I. NMR structure of the calponin homology domain of human IQGAP1 and its implications for the actin recognition mode. *J Biomol NMR* 2010; 48: 59-64


43 Wang W, Malcolm BA. Two-stage PCR protocol allowing...
introduction of multiple mutations, deletions and insertions using QuikChange Site-Directed Mutagenesis. Biotechniques 1999; 26: 680-682


45 Timson DJ, Trayner IP. The role of the proline-rich region in A1-type myosin essential light chains: implications for information transmission in the actomyosin complex. FEBS Lett 1997; 400: 31-36


---

S-Editor Cheng JX  L-Editor A  E-Editor Zhang DN