Characterization of the Interaction between RhoGDI and Cdc42Hs Using Fluorescence Spectroscopy*

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Tyzoom K. Nomanbhoy and Richard A. Cerione‡

From the Department of Biochemistry, Molecular and Cell Biology, and the Department of Pharmacology, Cornell University, Ithaca, New York 14853

The GDP-dissociation-inhibitor (GDI) for Rho-like GTP-binding proteins is capable of three different biochemical activities. These are the inhibition of GDP dissociation, the inhibition of GTP hydrolysis, and the stimulation of the release of GTP-binding proteins from membranes. In order to better understand how GDI interactions with Rho-like proteins mediate these different effects, we have set out to develop a direct fluorescence spectroscopic assay for the binding of the GDI to the Rho-like protein, Cdc42Hs. We show here that when the GDI interacts with Cdc42Hs that contains bound N-methylanthraniloyl GDP (Mant-GDP), there is an ~20% quenching of the Mant fluorescence. The GDI-induced quenching is only observed when Mant-GDP is bound to Spo0doptera frugiperda-expressed Cdc42Hs and is not detected when the Mant nucleotide is bound to Escherichia coli-expressed Cdc42Hs and thus shows the same requirement for isoprenylated GTP-binding protein as that observed when assaying GDI activity. A truncated Cdc42Hs mutant that lacks 8 amino acids from the carboxyl terminus and is insensitive to GDI regulation also does not show changes in the fluorescence of its bound Mant-GDP upon GDI addition. Thus, the GDI-induced quenching of Mant-GDP provides a direct read-out for the binding of the GDI to Cdc42Hs. Titration profiles of the GDI-induced quenching of the Mant-GDP fluorescence are saturable and are well fit to a simple 1:1 binding model for Cdc42Hs-GDI interactions with an apparent $K_d$ value of 30 nM. A very similar $K_d$ value (28 nM) is measured when titrating the GDI-induced quenching of the fluorescence of Mant-guananylyl imidodiphosphate, bound to Cdc42Hs. These results suggest that the GDI can bind to the GDP-bound and GTP-bound forms of Cdc42Hs equally well. We also have used the fluorescence assay for GDI interactions to demonstrate that the differences in functional potency observed between the GDI molecule and a related human leukemic protein, designated LD4, are due to differences in their binding affinities for Cdc42Hs. This, together with the results from studies using GDI/LD4 chimeras, allow us to conclude that a limit region within the carboxyl-terminal domain of the GDI molecule is responsible for its ability to bind with higher affinity (compared with LD4) to Cdc42Hs.

The Ras-like low molecular weight GTP-binding proteins form a superfamily whose members are involved in a plethora of biological pathways that include the regulation of cell growth and differentiation, vesicular transport, and cytoskeletal organization. The GTP-binding proteins appear to act as molecular switches by cycling between an inactive GDP-bound state and an active GTP-bound state. This cycle is tightly regulated by distinct proteins. In particular, the exchange of GDP to GTP is stimulated by guanine nucleotide exchange factors (GEFs), and the hydrolysis of GTP back to GDP is catalyzed by GTPase-activating proteins (GAPs). A third class of regulatory proteins were discovered based on their ability to inhibit GDP dissociation (and thus were originally designated as GDP-dissociation inhibitors or GDIs). The GDIs are capable of two other important biochemical activities, namely they inhibit GTP hydrolysis and promote the dissociation of GTP-binding proteins from membranes. Given these various activities, it has been speculated that the GDIs might play a critical role in mediating the movement of GTP-binding proteins between different cellular locations.

A major interest in our laboratory has been directed toward understanding the mechanism of regulation of the GTP-binding/GTPase cycle of the Cdc42Hs GTP-binding protein. Cdc42Hs is the human homolog of the Saccharomyces cerevisiae cell division cycle protein (Cdc42Sc) (Johnson and Pringle, 1990; Shinjo et al., 1990), which plays an essential role in the establishment of cell polarity by controlling the assembly of the bud site. The Schizosaccharomyces pombe Cdc42 protein is essential for both uni-directional and bi-directional cell growth (Miller and Johnson, 1994), and the mammalian Cdc42 protein has recently been implicated in filopodia formation (Kozma et al., 1995; Nobes and Hall, 1995) and in the stimulation of the nuclear kinases Jnk and p38 (Cos0 et al., 1995; Minden et al., 1995; Hill et al., 1995; Bagrodia et al., 1995a). Several regulators of the GTP-binding/GTPase cycle of Cdc42Hs have now been identified. These include the Db1 oncogene product (Hart et al., 1991a), which serves as a GEF for Cdc42 in mammalian cells, and the Cdc24 gene product, which functions as a GEF for Cdc42 in S. cerevisiae (Zheng et al., 1994). In addition, a specific GAP (designated the Cdc42Hs-GAP) has been purified (Hart et al., 1991b) and cloned (Barford et al., 1993), and a Cdc42Hs-GDI activity was purified from brain cytosol and shown to be identical to the RhoGDI (Leonard et al., 1992).

To better understand how these different proteins regulate the GTP-binding/GTPase cycle of Cdc42Hs, we have developed real time fluorescence spectroscopic assays to monitor each step of the cycle. Initially, we used the fluorescence of the single

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‡To whom correspondence should be addressed. Tel.: 607-253-3888; Fax: 607-253-3639.

1 The abbreviations used are GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein for low molecular mass GTP-binding proteins; GDI, GDP-dissociation inhibitor; GppNp, guanosine 5-(p)-imidotriphosphate; GST, glutathione S-transferase; Mant, N-methylanthraniloyl; CHAPS, 3-([3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.
tryptophan residue of Cdc42Hs (Trp279) as an intrinsic reporter group for monitoring GTP hydrolysis (Leonard et al., 1994). In addition, along the lines of experiments performed with Ras (Antony et al., 1991; Rensland et al., 1991; Moore et al., 1993), we have used the fluorescence of Mant-derivatized nucleotides to monitor the kinetics of nucleotide binding and dissociation from Cdc42Hs (Leonard et al., 1994). More recently, we have covalently attached an exogenous fluorescent probe to Cdc42Hs and used the fluorescence of the probe to directly monitor the GTP-binding and GTPase activities of Cdc42Hs (Nomanbhoy et al., 1996).

Although the methods discussed above have provided us with real time read-outs for the GEF-catalyzed GDP-GTP exchange and GAP-stimulated GTP hydrolytic reactions, they have not provided a means to directly monitor the binding of Cdc42Hs to its GDI (i.e. the RhoGDI). There is a great deal of interest in understanding the interactions of RhoGDI with Cdc42Hs and related proteins (i.e. Rac and RhoA) because of the multiple regulatory activities exhibited by the GDI, i.e. its ability to bind to both the GDP- and GTP-bound forms of Cdc42Hs and to elicit the release of both forms of Cdc42Hs from membranes. As a first step toward characterizing these interactions, we set out to develop a fluorescence assay that would allow us to directly monitor Cdc42Hs-RhoGDI complex formation in real time. In the present study, we describe such a read-out that takes advantage of a GDI-induced quenching of the fluorescence of Mant-nucleotides bound to Cdc42Hs. We have gone on to use this read-out to address two important questions regarding the actions of RhoGDI. The first concerns the relative ability of RhoGDI to bind to the GDP- and GTP-bound forms of Cdc42Hs. This question directly bears on the relevance of RhoGDI as a GTPase inhibitor of Cdc42Hs and on the ability of RhoGDI to cause activated (GTP-bound) forms of Cdc42Hs to be released from membranes. The second question that we have addressed relates to the mechanistic basis of the functional differences that have been observed between RhoGDI and a highly related molecule, designated LD4, in their actions toward Cdc42Hs. Specifically, it has been shown that although LD4 has GDI activity toward Cdc42Hs, it is significantly less potent than that of the RhoGDI. Recent chimera studies have demonstrated that the carboxyl-terminal domains of the RhoGDI and LD4 molecules are responsible for the difference in potency (Platko et al., 1995). In the present study, we have used the fluorescence read-out for GDI interactions to determine if the functional differences between RhoGDI and LD4 directly reflect differences in their binding affinity for Cdc42Hs. If so, this then would highlight the amino acid residues on the RhoGDI molecule that are critical for high affinity binding to GTP-binding proteins.

**EXPERIMENTAL PROCEDURES**

**Purification of RhoGDI, RhoGDI-L8, LD4, and LDGDI—**RhoGDI, RhoGDI-L8, LD4, and Chimeras C, B, and D were purified as GST fusion proteins from Escherichia coli. The dicing and expression of these proteins have been described (Platko et al., 1995). Briefly, a 5-mL overnight culture of E. coli containing the appropriate expression plasmid was used to seed 1 liter of superbroth (0.032 mg/ml bactotryptone, 0.02 mg/ml yeast extract, 0.005 mg/ml NaCl, pH 7.4). This was grown in a standard shaker at 37°C. Protein expression was induced at an A600 nm of ~0.6 with 200 μM isopropyl β-D-thiogalactoside for 60 min. Cells were harvested by centrifugation, quick-frozen in liquid nitrogen, and stored at ~80°C. Pellets were thawed into Buffer A (20 mM Tris pH 8.0, 50 mM EDTA, 0.2 mM phenylmethanesulfonyl fluoride, and 10 μM γ-mercaptoethanol and leupeptin) to which was added 1 mg/ml lysozyme. When lysis was complete, DNase was added to 0.05 mg/ml and MgCl2 was adjusted to 2 mM. The lysate was cleared by centrifugation, and the supernatant was incubated with glutathione-agarose beads (Sigma) (~300 μl agarose/15 ml lysate) for 15 min at 4°C. The beads were washed extensively with Buffer B (20 mM Tris, pH 8.0, 1 mM EDTA, 50 mM NaCl), and the GST fusion protein eluted with Buffer B supplemented with 10 mM glutathione. The concentration of protein was determined using the Bradford assay (Bradford, 1976).

**Fluorimetry of Isoprenylated Cdc42Hs from Spodoptera frugiperda Insect Cells—**Cdc42Hs was expressed as a GST fusion protein in S. frugiperda (S9) insect cells by infecting the cells with the appropriate recombinant baculovirus. Generally, infection was carried out for 72 h, and infected cells were harvested by centrifugation. Lysates from infected Cdc42Hs was purified by phase extraction with Triton-X 114. Cell pellets were lysed into 1× TBS, 10% Triton-X 114, 200 μM phenylmethanesulfonyl fluoride, and 10 μM γ-mercaptoethanol and leupeptin. The lysate was homogenized using a Dounce homogenizer and spun at 12,000 rpm for 10 min at 4°C in a microfuge. The supernatant from this spin was collected, warmed to 37°C for 2 min, and spun at 12,000 rpm for 2 min in a microfuge at room temperature. The lower detergent phase containing isoprenylated Cdc42Hs was pooled and diluted into 1× TBS so that the final detergent concentration was 1%. This was incubated with glutathione-agarose beads (Sigma) (~300 μl of agarose/10 ml of lysate) for 25 min at 4°C. The beads were washed extensively with Buffer B containing 0.1% CHAPS. GST-Cdc42Hs was then eluted from the column using Buffer C (50 mM Tris, pH 8.3, 150 mM NaCl, 5 mM MgCl2, 2.5 mM CaCl2, 0.1% CHAPS, 10 mM glutathione). 8 μl of a 1 unit/ml thrombin solution (Sigma) were added to the eluate, and thrombin digestion was carried out for 90 min at room temperature. Protein concentration was determined using the Bradford assay, and the percentage of isoprenylated Cdc42Hs in each preparation was estimated by performing a GDI assay using radiolabeled nucleotides (Leonard et al., 1992). The value generally ranged from 40 to 60%

**Preparation of Mant Nucleotide-labeled Cdc42Hs—**Mant-GDP and Mant-GppNp were synthesized from the parent nucleotides and N-methylisatoic acid according to the published procedure (Hiratsuka, 1983). Isoprenylated GST-Cdc42Hs, prepared as described above, was bound to glutathione-agarose beads. This was incubated in 10 mM EDTA and 20 μM Mant-GDP or Mant-GppNp for 20 min at room temperature. MgCl2 was then added to 20 mM to block the further exchange of Mant-nucleotide onto GST-Cdc42Hs. The beads were washed with Buffer B containing 0.1% CHAPS and 5 mM MgCl2 to remove free Mant-nucleotide. GST-Cdc42Hs labeled with Mant nucleotide eluted from the column using glutathione, and then Cdc42Hs was released from GST through thrombin digestion, as described above.

**Fluorescence Spectroscopy—**The fluorescence measurements were made using an SLM 8000c spectrofluorometer operated in the photon-counting mode. Samples were stirred continuously and thermostated at 30°C in Buffer D (20 mM Tris, pH 8.0, 50 mM NaCl, 2 mM MgCl2). Calculation of Dissociation Constants (Kd)—The Kd values for the binding of RhoGDI, LD4, and LD4/LD4 chimeras to Cdc42Hs-Mant-GDP, and RhoGDI to Cdc42Hs-Mant-GppNp were obtained using the following equation:

\[
F = F_0 + F_i \left[ 1 + \left( K_o + R_T \right)^2 \right] \left( 1 - K_d \left( 1 + R_T \right)^2 \right) \left( 4R_T^2 \right) \left( 1 - R_T \right) \]  

where \( F \) is the change in fluorescence over initial fluorescence \((\Delta F/F_0)\), \( F_i \) is the initial value for \((\Delta F/F_0)\), \( K_o \) is the total concentration of RhoGDI, LD4, or the chimeras, and \( R_T \) is the total concentration of Cdc42Hs-Mant-GDP or Cdc42Hs-Mant-GppNp. Fits were generated using IgorPro waveforms software.

**RESULTS**

The Mant Fluorescence of Isoprenylated Cdc42Hs-Mant-GDP Is Sensitive to RhoGDI Binding—A primary aim of these studies was to develop a fluorescence read-out that would enable us to directly monitor the interactions of RhoGDI with Cdc42Hs in real time. Because one of the key activities of the GDI is to block the dissociation of guanine nucleotide from the GTP-binding protein, we examined whether RhoGDI binding to Cdc42Hs influenced the microenvironment of the nucleotide binding site, as might be reflected by a change in the fluorescence of Mant nucleotides. The results presented in Fig. 1A show that this is in fact the case. Specifically, the addition of RhoGDI to the Cdc42Hs-Mant-GDP complex results in a ~20% decrease in Mant fluorescence. It should be noted that the emission maxima for both spectra (i.e. in the presence and the absence of added RhoGDI) are about the same (~445 nm). This indicates that the conformational change in the vicinity of the GTP-binding site, as might be reflected by a change in the fluorescence of Mant nucleotides. The results presented in Fig. 1A show that this is in fact the case. Specifically, the addition of RhoGDI to the Cdc42Hs-Mant-GDP complex results in a ~20% decrease in Mant fluorescence. It should be noted that the emission maxima for both spectra (i.e. in the presence and the absence of added RhoGDI) are about the same (~445 nm). This indicates that the conformational change in the vicinity of the
Mant moiety does not involve a significant change in the hydrophobicity of the environment surrounding the nucleotide, as might be expected if the fluorescence change were an outcome of the GDI molecule directly covering the nucleotide site. Rather, these results suggest that RhoGDI binding to Cdc42Hs induces a conformational change that brings a "quenching residue" into the vicinity of the Mant moiety.

As shown in Fig. 1B, the interaction between RhoGDI and Cdc42Hs that gives rise to the quenching of Mant-GDP fluorescence is rapid and occurs within the time period of mixing (<5 s). The RhoGDI-induced decrease in Mant-GDP fluorescence is also specific for isoprenylated Cdc42Hs, consistent with earlier suggestions that the geranyl-geranylation of Rho subtype GTP-binding proteins is necessary for their interactions with GDI (Ueda et al., 1990). No quenching of the Mant-GDP fluorescence was observed when RhoGDI was added to Cdc42Hs that was expressed and purified from E. coli or when added to Cdc42Hs that was expressed in Sf9 cells but purified from the aqueous fraction of the Triton-X 114 phase extraction (data not shown).

There were a number of other criteria that would be expected to be met if the RhoGDI-induced quenching of the Mant fluorescence reflected a direct interaction between GDI and Cdc42Hs. For example, it would be expected that the addition of isoprenylated (Sf9-expressed) Cdc42Hs-GDP to the Mant-GDP would compete with the Cdc42Hs-Mant-GDP complex and thus block the RhoGDI-induced change in Mant fluorescence. As shown in Fig. 2A, the inclusion of excess isoprenylated GDP-bound Cdc42Hs (250 nM) prior to the addition of RhoGDI completely inhibited the GDI-dependent quenching of the Mant fluorescence. This inhibition was specific for isoprenylated Cdc42Hs, because the addition of nonisoprenylated (E. coli-expressed) Cdc42Hs to 450 nM only slightly attenuated the quenching of Mant fluorescence by RhoGDI (Fig. 2B). In addition, when RhoGDI was first added to the Cdc42Hs-Mant-GDP complex, in order to effect a quenching of the Mant fluorescence, it was possible to rapidly reverse the GDI-induced quenching by subsequently adding an excess of isoprenylated GDP-bound Cdc42Hs (Fig. 2C).

Finally, in a previous study, we characterized a RhoGDI truncation mutant in which 8 amino acids had been removed from the carboxyl terminus (RhoGDI Δ8). Specifically, we observed that RhoGDI Δ8 showed absolutely no GDI activity toward Cdc42Hs (Platko et al., 1995). This indicated that the carboxyl-terminal domain of RhoGDI contains residues that are critical for the interaction between RhoGDI and Cdc42Hs, either through a direct involvement in binding Cdc42Hs or through the maintenance of an appropriate tertiary structure. We would therefore predict that due to its inability to bind to Cdc42Hs, RhoGDI Δ8 would not induce a quenching of the Mant fluorescence of Cdc42Hs-Mant-GDP. Fig. 3 shows that this
turned out to be the case, that is the addition of RhoGDI to isoprenylated Cdc42Hs-Mant-GDP did not cause a significant decrease in Mant fluorescence. Thus, taken together, our observations in Figs. 1–3 indicate that the quenching of the Mant fluorescence by RhoGDI is a direct reflection of the binding of GDI to Cdc42Hs.

**Direct Determination of the Binding Affinity for the RhoGDI/Cdc42Hs-Mant-GDP Interaction**—Having established a direct fluorescence assay for the binding of RhoGDI to Cdc42Hs, we then used this read-out to determine the affinity of the GDI molecule for the Mant-GDP-bound form of Cdc42Hs. Fig. 4A shows the results of an experiment where the level of the Cdc42Hs-Mant-GDP complex was kept constant and increasing amounts of the RhoGDI were added to the fluorescence cuvette while continuously monitoring the emission of Mant fluorescence at 440 nm. The raw data show that the RhoGDI-induced quenching is a saturable response. Fig. 4B shows the resultant titration profile obtained from the data; the titration data can be fit well to a simple binding model (solid line) that assumes a single class of binding sites for the interaction between Mant-GppNp-Cdc42Hs and the GDI. The calculated dissociation constant (K_d) value for the interaction is 28 nM, which is virtually identical to that for the interaction between RhoGDI and Mant-GDP-bound Cdc42Hs. Thus, these results indicate that the RhoGDI binds equally well to both nucleotide states of Cdc42Hs.

**Comparison of the Binding of RhoGDI and the Related Human Leukemia Protein, LD4, with Cdc42Hs**—We have characterized the hematopoietic cell homolog for GDI, LD4, and observed that despite having significant sequence similarity to RhoGDI (67% identity), the activity of LD4 toward Cdc42Hs is about 10–20-fold weaker than that of RhoGDI (Adra et al., 1993; Platko et al., 1995). One possibility for this difference is that the LD4 protein binds to Cdc42Hs with a much weaker affinity than does the RhoGDI. If this is the case, it would have important implications regarding the site on RhoGDI that is responsible for conferring high affinity binding to Cdc42Hs, because we recently have mapped the site on RhoGDI that is responsible for its enhanced functional potency (relative to LD4) to a region within the carboxyl-terminal domain of the molecule (see below; see also Platko et al. (1995)). We therefore measured the binding of the recombinant LD4 molecule to Cdc42Hs using the direct fluorescence read-out. The addition of LD4 to Mant-GDP-bound Cdc42Hs resulted in a quenching of the Mant-GDP fluorescence, just as was the case with RhoGDI. However, as shown in Fig. 6 (squares), LD4 binds to the Cdc42Hs-Mant-GDP complex with a significantly weaker affin-
Fluorescence Studies of Cdc42Hs-GDI Interactions

A chimera that contained the first 171 amino acids from RhoGDI, as well as residues 172–180 (from the carboxyl-terminal 33 residues), and the last 24 residues from LD4 (designated Chimera C) also was fully active toward Cdc42Hs, whereas a chimera that contained the first 171 amino acids from RhoGDI, followed by 8 amino acids from LD4, and then the final 25 amino acids from RhoGDI (designated as Chimera D) behaved like wild type LD4. Fig. 6 shows an analysis of the binding of Chimeras B (triangles), C (diamonds), and D (inverted triangles) to Cdc42Hs-Mant-GDP. The binding profiles obtained with these chimeras exactly corresponded to the results obtained in functional assays (Platko et al., 1995). From the best fits to the binding data, we determined $K_d$ values of 20 nM for Chimera B, 23 nM for Chimera C, and 380 nM for Chimera D. These results strongly argue that the differences in functional potency between RhoGDI and LD4 toward Cdc42Hs are a direct outcome of the different binding affinities of these regulatory proteins.

**DISCUSSION**

A long term goal of our laboratory has been to develop fluorescence spectroscopic read-outs to aid in the biochemical characterization of different proteins that regulate the GTP-binding/GTPase cycle of Cdc42Hs. An important aim was to develop such a read-out for the RhoGDI molecule, because it is capable of a number of interesting regulatory activities and because we already had obtained a good deal of structure-function information from chimera studies of the GDI and a related molecule, LD4 (Platko et al., 1995). In the present work, we describe a real time spectroscopic read-out for RhoGDI interactions with Cdc42Hs bound to the fluorescent nucleotide, Mant-GDP. We show that as an outcome of RhoGDI binding, a significant quenching (~20%) of the Mant-GDP fluorescence occurs. The RhoGDI-induced quenching shows all the characteristics expected for a proper interaction between this regulator and the GTP-binding protein, namely that it is specific for the isoprenylated form of Cdc42Hs and is not detected when using a carboxyl-terminal truncated mutant of the GDI, which was previously shown to be incapable of binding.

Using this fluorescence assay, one of the important questions that we set out to address was how the binding of the RhoGDI to the GTP-bound (activated) form of Cdc42Hs compared with its binding to the GDP-bound (inactive) form. We found that these interactions occur with virtually identical affinities, thus suggesting that the ability of the RhoGDI to bind to the Cdc42Hs-GTP species and inhibit the GTPase activity may be as important (in terms of biological regulation) as the previously recognized binding of this regulator to the Cdc42Hs-GDP complex. One possibility is that the RhoGDI interaction is essential for “shuttling” Cdc42Hs between different membrane locations within the cell. We recently have demonstrated that a predominant location of the GDP-bound form of Cdc42Hs is the Golgi and that this location is influenced by the Arf GTP-binding protein, which has been implicated in intracellular trafficking. These findings, together with the biological effects that Cdc42Hs mediates at the plasma membrane (filopodia formation) (Nobes and Hall, 1995; Kozma et al., 1995), suggest that the GTP-binding protein may need to cycle between these different membrane locations. Thus, the ability of the RhoGDI to bind equally well to both the GDP-bound and GTP-bound forms of Cdc42Hs, coupled with its ability to release Cdc42Hs from membranes (Leonard et al., 1992), may enable this regulatory protein to facilitate the movement of the appropriate protein to its target.
nucleotide-bound form of Cdc42Hs to the proper membrane target protein. However, it also is possible that as yet unidentified cellular proteins influence the interactions between Cdc42Hs and the RhoGDI and either enhance or inhibit its ability to bind to specific nucleotide states of Cdc42Hs.

We have found that we can also use this fluorescence assay to directly monitor the binding of the human leukemia protein, LD4, to Cdc42Hs. The LD4 protein shows a relatively high degree of sequence identity to RhoGDI, with the sequence homology extending throughout the entire length of these molecules. Interestingly, however, we have shown that LD4 is much less effective as a GDI toward Cdc42Hs, being 10–20-fold less potent. These differences were first narrowed down to the carboxyl-terminal 33 amino acid residues of the two molecules, based on studies using GDI/LD4 chimeras to assay GDI activity toward Cdc42Hs (Platko et al., 1995). It was then shown that these differences could be attributed to as few as six amino acid differences that existed between residues 172–180 of RhoGDI and the corresponding residues 169–178 of LD4. Moreover, a single amino acid change at residue 174 of LD4 to the corresponding residue of RhoGDI could impart nearly full GDI activity to the LD4 molecule. Our present fluorescence studies now show that the differences in functional potency between the RhoGDI and LD4 can be entirely attributed to differences in their abilities to bind to Cdc42Hs. This then argues that residues 172–180 of the RhoGDI molecule are responsible for the ability of this regulatory protein to bind with high affinity to the GTP-binding protein. Because two of these residues are conserved in LD4, these results argue that as few as six amino acids are responsible for the significantly higher affinity of RhoGDI (compared with LD4) for Cdc42Hs. At present, we still do not know the identity of all of the amino acid residues on the GDI molecule and Cdc42Hs that are critical for the binding interaction between these proteins. However, we intend to use this read-out to screen a variety of mutants of GDI and the GTP-binding protein to gain a more complete picture of the contact sites that are involved. We also hope to use this read-out in conjunction with fluorescence resonance energy transfer measurements in membrane preparations to address the molecular mechanism that underlies the GDI-stimulated release of Cdc42Hs from phospholipid bilayers.

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REFERENCES

Adra, C. N., Ko, J., Leonard, D., Wirth, L. J., Cerione, R. A., and Lim, B. (1993) Gene Chromosomes Cancer 8, 253–261
Antonny B., Chardin, P., Roux, M., and Chabre M. (1991) Biochemistry 30, 8287–8295
Bagrodia, S., Dérijard, B., Davis, R. J., and Cerione, R. A. (1995a) J. Biol. Chem. 270, 27995–27998
Bagrodia, S., Taylor, S. J., Creasy, C. L., Chernoff, J., and Cerione, R. A. (1995b) J. Biol. Chem. 270, 22731–22737
Barford, E. T., Zheng, Y., Kuang, W., Hart, M. J., Evans, T., Cerione, R. A., and Ashkenazi, A. (1993) J. Biol. Chem. 268, 26059–26062
Bradford, M. M. (1976) Anal Biochem. 72, 248–254
Coso, O. A., Chiariello, M., Yu, J., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1990) Cell 81, 1137–1146
Hart, M. J., Eva, A., Evans, T., Aaronson, S. A., and Cerione, R. A. (1991a) Nature 354, 314–317
Hart, M. J., Shinjo, K., Hall, A., Evans, T., and Cerione, R. A. (1991b) J. Biol. Chem. 266, 20840–20848
Hart, M. J., Morv, Y., Leonard, D., Witte, O. N., Evans, T., and Cerione, R. A. (1992) Science 258, 812–815
Hill, C. S., Wynne, J., and Treisman, R. (1995) Cell 81, 1159–1170
Hiratsuka, T. (1983) Biochim Biophys. Acta 742, 496–508
Holl, D. I., and Pringle, J. R. (1990) J. Cell Biol. 111, 143–152
Kozma, K., Ahmend, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15(4), 1942–1952
Leonard, D., Hart, M. J., Platko, J. V., Eva, A., Henzel, W., Evans, T., and Cerione, R. A. (1992) J. Biol. Chem. 267, 22860–22868
Leonard, D. A., Evans, T., Hart, M., Cerione, R. A., and Manor, D. (1994) Biochemistry 33, 12323–12328
Miller, P., and Johnson, D. I. (1994) Mol. Cell. Biol. 14, 1075
Nobes, C. D., and Hall, A. (1996) Cell 84, 53–62
Norrer, A., Lautwein, A., Wittinghofer, A., and Goody, R. S. (1991) Biochemistry 30, 11181–11185
Sasaki, T., Kituchi, A., Araki, S., Hata, Y., Isomura, M., Kurodo, S., and Takai, Y. (1990) J. Biol. Chem. 265, 2333–2337
Shinjo, K., Ko, J., Hart, M. J., Narasimhan, V., Johnson, D. I., Evans, T., and Cerione, R. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9853–9857
Ueda, T., Kituchi, A., Ohgi, N., Yamamoto, J., and Takai, Y. (1995) J. Biol. Chem. 265, 9373–9380
Zheng, Y., Cerione, R., and Bender, A. (1994) J. Biol. Chem. 269, 2369–2372
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