Regulation of Cross-linking of Actin Filament by IQGAP1, a Target for Cdc42*

(Received for publication, July 7, 1997, and in revised form, September 4, 1997)

Masaki Fukata§, Shinya Kuroda¶, Katsuhiro Fujii‡, Tomoko Nakamura‡, Ikuo Shoji§, Yoshiharu Matsura†, Katsuya Okawa**, Akihiro Iwamatsu**, Akira Kikuchi†, and Kozo Kaibuchi‡‡

From the Department of Signal Transmission, Nara Institute of Science and Technology, Ikoma 630-01, the Department of Biochemistry, Hiroshima University School of Medicine, Hiroshima 734, the Inheritance and Variation Group, Precursory Research for Embryonic Science and Technology, Kyoto 619-02, the Department of Virology II, National Institute of Infectious Diseases, Tokyo 162, and the **Central Laboratories for Key Technology, Kirin Brewery Company, Ltd., Yokohama 236, Japan

We have previously shown that IQGAP1, a recently identified target for Cdc42 and Rac1 small GTPases, showed a distribution similar to that of cortical actin cytoskeleton at the membrane ruffling area induced by insulin and Rac1val12 (Kuroda, S., Fukata, M., Kobayashi, K., Nakafuku, M., Nomura, N., Iwamatsu, A., and Kaibuchi, K. (1996) J. Biol. Chem. 271, 23363–23367). Here we identified an IQGAP1-interacting molecule with molecular mass of 43 kDa (p43) from bovine brain cytosol, using glutathione S-transferase (GST–IQGAP1) affinity column chromatography. The amino acid sequencing of the protein revealed that p43 was identical to β- and γ-actin. IQGAP1 was cosedimentated with filamentous actin (F-actin). The amino-terminal domain (amino acids 1–216) of IQGAP1 was responsible for the interaction with F-actin. Falling ball viscometry assay revealed that IQGAP1 cross-linked the F-actin. This IQGAP1 activity was further enhanced by guanosine 5'-O-(3-thiotriphosphate) (GTPγS)-GST-Cdc42 but not by GDP–GST–Cdc42. The gel filtration analysis of IQGAP1 revealed that IQGAP1 appeared as oligomers and that GTPγS–GST–Cdc42 but not GDP–GST–Cdc42 enhanced the oligomerization of IQGAP1. These results strongly suggest that IQGAP1, acting downstream of Cdc42, can cross-link the actin filament through its oligomerization.

Cdc42 and Rac1, the members of Rho small GTPases, are shown to regulate a variety of morphological events through actin cytoskeleton (for reviews see Refs. 1 and 2). Cdc42 and Rac1 are implicated in the filopodia (3, 4) and lamellipodia (4, 5) formation, respectively, in Swiss 3T3 cells. Target molecules for Cdc42 and Rac1 have thus far been identified to be serine/threonine kinase PAK (6–8), WASP1 (9, 10), and IQGAP1 (10, 16). Despite of these intensive studies, how Cdc42 and Rac1 regulate actin cytoskeleton is still unclear. We have previously identified IQGAP1 as a target for Cdc42 and Rac1 and found that it was colocalized with the actin filament at the lamellipodia induced by insulin and Rac1val12 (14). This result led us to examine the role of IQGAP1 as a regulator of actin cytoskeleton.

IQGAP1 has a so called CHD (13, 17), which is also present in several actin-binding proteins, including calponin (18), filamin (19), α-actinin (20), and fimbrin (21). On the other hand, filamin (22), α-actinin (20), and fimbrin (23) are known to cross-link the actin filament (24, 25). It has been shown that oligomerization of filamin or α-actinin enables them to cross-link the F-actin. Cross-linking of F-actin appears to be important for the filopodia and lamellipodia formation. However, little is understood about how cross-linking of F-actin is regulated by intracellular signals.

In this study, we identified actin as an IQGAP1-interacting molecule and found that IQGAP1 interacted with F-actin in vitro and cross-linked F-actin in a GTPγS–GST–Cdc42-dependent manner. We also showed here that IQGAP1 formed oligomers, suggesting that IQGAP1 cross-links the actin filament through its oligomerization.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—Anti-actin polyclonal antibody was kindly provided by Dr. I. Yahara (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan). Anti-IQGAP1 polyclonal antibody was raised against GST–IQGAP1 (aa 1–863) as an antigen. All materials used in the nucleic acid study were purchased from Takara Shuzo Co. (Kyoto, Japan). Other materials and chemicals were obtained from commercial sources.

Preparation of Recombinant Protein—Myc–IQGAP1 (aa 1–1657) and GST–IQGAP1 (aa 1–1657) were purified from overexpressing Spodoptera frugiperda insect cells. The insect cells overexpressing Myc–IQGAP1 were homogenized with Buffer A (20 mM Tris/HCl at pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, and 10 μM (p-aminodiphenyl)-methane-sulfonil fluoride on ice and centrifuged at 100,000 × g for 1 h at 4 °C. The extracts were centrifuged at 100,000 × g for 1 h at 4 °C. Then Myc–IQGAP1 was purified from the supernatant by MonoQ column chromatography. For falling ball viscometry assay, GST–IQGAP1 was purified by glutathione-Sepharose column chromatography from overexpressing...
insect cells. For gel filtration chromatography, the insect cells overexpressing Myc-IQGAP1 were homogenized with Buffer A on ice and centrifuged at 100,000 \( \times g \) for 1 h at 4 °C. The supernatant was loaded onto calmodulin-Sepharose 4B column. After washing the column with Buffer A containing 500 mM NaCl, Myc-IQGAP1 was eluted with Buffer A containing 2 mM NaCl. The purity of Myc-IQGAP1 in the eluate was about 95% estimated by SDS-PAGE.

**GST-IQGAP1 Affinity Column Chromatography**—The affinity purification was performed essentially as described (14, 26). Briefly, bovine brain cytosol was passed through glutathione beads to remove endogenous GST. Then the pass fraction was loaded on glutathione beads containing GST, GST-IQGAP1-N-1 (aa 1–1657), N-2 (aa 1–216), N-3 (aa 216–683), M-1 (aa 521–914), or C-1 (aa 764–1252) as described (14, 26). After washing the columns, bound proteins were eluted by the addition of Buffer A containing 500 mM NaCl.

**Cosedimentation Assay**—F-actin was purified from an aceton powder prepared from rabbit skeletal muscle as described (27). F-actin was mixed with either Myc-IQGAP1 (aa 1–1657), GST-IQGAP1-N-1 (aa 1–863), GST-IQGAP1-N-2 (aa 1–216), GST-IQGAP1-N-3 (aa 216–683), or GST-IQGAP1-M-1 (aa 521–914) and incubated at room temperature for 1 h with or without either GST, GDP-GST-Cdc42, or GTP-\( \gamma \)-S-GST-Cdc42 (3 \( \mu \)M each) in Buffer B (20 mM Tris/\( \text{HCl} \) at pH 7.4, 0.5 mM dithiothreitol, 2 mM MgCl\(_2\), 100 mM NaCl, 1 mM EDTA, 10% (w/v) sucrose, 0.5 mM ATP, 10 \( \mu \)g/ml leupeptin, and 10 \( \mu \)M (p-amidinophenyl)-methylene-bis-(4-nitrophenyl) fluorophore). After the incubation, 50 \( \mu \)l of each reaction mixture was layered onto a 100-\( \mu \)l sucrose barrier (20% (w/v) sucrose in 300 mM NaCl). Aliquots of the eluates were resolved by SDS-PAGE followed by silver staining. The arrow and arrowhead denote the positions of p43 and p41, respectively. B, aliquots of the eluates were resolved by SDS-PAGE followed by Western blotting with anti-actin antibody. The arrow denotes the position of actin. The results shown are representative of three independent experiments.

**DDAPRAVPF**, both of which are identical to those of \( \beta \)- and \( \gamma \)-actin. The molecular weights of \( \beta \)- and \( \gamma \)-actin were calculated to be 41,605 and 41,661, respectively, which are almost the same as that of p43. We also confirmed that p43 was recognized by anti-actin antibody (Fig. 1B). Therefore, we concluded that p43 was actin. Identification of p41 is currently under investigation.

IQGAP1 has a CHD (17), which is also present in several actin-binding proteins, including calponin, filamin, \( \alpha \)-actinin, and fimbrin, in its amino terminus (13, 32). To determine whether IQGAP1 directly interacts with F-actin, cosedimentation assay of recombinant IQGAP1 with F-actin was performed. Myc-IQGAP1 was cosedimented in the presence of F-actin but not in the absence of F-actin (Fig. 2A), indicating that IQGAP1 directly interacts with F-actin. GST-IQGAP1-N-1 (aa 1–863) was also cosedimented with F-actin, whereas IQGAP1-C-1 (aa 764–1252) was not (data not shown). We produced the indicated mutants of IQGAP1, and their interactions with F-actin were assessed. GST-IQGAP1-N-2 (aa 1–216) was cosedimented with F-actin, whereas others were not (Fig. 2B). Therefore, the amino terminus of IQGAP1 (aa 1–216) containing the CHD is sufficient for the F-actin binding. To examine whether Cdc42 affects this binding, we used Myc-IQGAP1 (aa 1–1657) because Cdc42 does not interact with IQGAP1-N-1 (aa 1–863) but interacts with Myc-IQGAP1 (aa 1–1657) (14). The similar assay was performed in the presence of a GDP-\( \gamma \)-S-GST-Cdc42 or GDP-GST-Cdc42. Neither GTP-\( \gamma \)-S-GST-Cdc42 nor GDP-GST-Cdc42 affected the F-actin binding activity of Myc-IQGAP1 (aa 1–1657) (data not shown). We also examined whether IQGAP1 interacts with globular actin using a GST-IQGAP1-N-1 (aa 1–863) affinity column chromatography and found that IQGAP1 did not interact with globular actin (data not shown).

Next we examined whether IQGAP1 cross-links the F-actin. Actin-based viscosity was measured by the falling ball viscometry assay (29). Actin-based viscosity was markedly increased in the presence of GST-IQGAP1 (aa 1–1657) and GST-IQGAP1-N-1 (aa 1–863) in a dose-dependent manner compared with

---

2 Identification of p41 will be described elsewhere (study in progress).
that in the absence of IQGAP1 (Fig. 3A), suggesting that IQGAP1 cross-links F-actin. The shorter fragments, GST-IQGAP1-N-2 (aa 1–216), GST-IQGAP1-N-3 (aa 216–683), and GST-IQGAP1-M-1 (aa 521–914), did not increase the viscosity. We examined whether Cdc42 affects this activity. The similar assay was performed in the presence of the absence of GST, GTPγS-GST-Cdc42, or GDP-GST-Cdc42. GTPγS-GST-Cdc42 markedly enhanced the cross-linking activity of GST-IQGAP1 (aa 1–1657), whereas GDP-GST-Cdc42 did not (Fig. 3B). GTPγS-GST-Cdc42 did not enhance the cross-linking activity of GST-IQGAP1 (aa 1–863) because Cdc42 did not interact with IQGAP1-N-1 (aa 1–863) (14) (data not shown).

When bovine brain cytosol was subjected to the indicated mutants of IQGAP1 affinity column chromatography, endogenous IQGAP1 was specifically detected in the GST-IQGAP1-N-3 (aa 216–683) eluate (Fig. 4A). IQGAP1 was not detected in the GST, GST-IQGAP1-N-2 (aa 1–216), GST-IQGAP1-M-1 (aa 521–914), and IQGAP1-C-1 (aa 764–1657) eluates. This result suggests that IQGAP1 forms oligomers through at least the amino-terminal portion of IQGAP1 (aa 216–683). We examined whether guanine nucleotides or Cdc42 affect this interaction. A similar assay was performed in the presence or the absence of GDP, GTPγS, GDP-Cdc42, or GTPγS-Cdc42. None of these conditions affected this interaction (Fig. 4B). Actin-cross-linking proteins, such as filamin (19) and α-actinin (33), form oligomers, and the oligomerization enables them to cross-link F-actin (25). Therefore, we examined whether IQGAP1 can form oligomers by gel filtration analysis. IQGAP1 appeared as broad major and minor peaks, corresponding to the molecular masses of about 300 and 500 kDa, respectively (Fig. 5). These peaks may contain monomers, dimers, and trimers, suggesting that IQGAP1 can form oligomers. GTPγS-GST-Cdc42 but not GDP-GST-Cdc42 markedly shifted the peak of IQGAP1, corresponding to the molecular mass of about 600 kDa. GTPγS-GST-Cdc42 alone was eluted as a dimer. Judging from the molecular mass of a dimer of GTPγS-GST-Cdc42 (about 100 kDa), it is likely that GTPγS-GST-Cdc42 enhances the oligomerization of IQGAP1. These results strongly...
GTP-γS-GST-Cdc42 enhanced this IQGAP1 activity when 300 nM of IQGAP1 was used, although Cdc42 did not affect the activity of IQGAP1 when more than 750 nM of IQGAP1 was used. This result suggests that Cdc42 caused a leftward shift of the curve of the IQGAP1 activity. The concentration of endogenous IQGAP1 was calculated to be about 300 nM in cultured cells, such as MTD-1A epithelial cells (data not shown). At this concentration of IQGAP1, Cdc42 can further enhance the cross-linking activity of IQGAP1. Therefore, it is plausible that IQGAP1 physiologically cross-link the actin filament in a Cdc42-dependent manner in vivo.

GTP-γS-Cdc42 did not affect the interaction of IQGAP1 with F-actin, whereas GTP-γS-Cdc42 enhanced the oligomerization of IQGAP1. This may account for how Cdc42 induces the cross-link of the actin filament through IQGAP1. The mechanism by which GTP-γS-Cdc42 elicits the oligomerization of IQGAP1 is not known at present. A possible explanation is that GTP-γS-Cdc42 affects the conformation of IQGAP1 and subsequently promotes the oligomerization of IQGAP1 through the amino-terminal portion (aa 216–683) of IQGAP1. However, this possibility may be less likely because neither GTP-γS nor GTP-γS-Cdc42 affected the interaction of bovine IQGAP1 with the amino-terminal portion (aa 216–683) of IQGAP1. Alternatively, Cdc42 itself may make oligomers so that IQGAP1-Cdc42 complex consequently forms additional oligomers. It has been shown that Ras and RhoA, members of small GTPases, form oligomers (36, 37). If Cdc42 forms oligomers as in the case of Ras and RhoA, this possibility is likely. However, a further study to address this issue is necessary.

Very recently, during the revision of this manuscript, IQGAP1 has been shown to bind F-actin and cross-link F-actin (38). Our result is consistent with this observation. We showed here that GTP-γS-GST-Cdc42 enhanced the F-actin-cross-linking activity of IQGAP1, possibly through enhancing the oligomerization of the IQGAP1-Cdc42 complex. Therefore, IQGAP1 appears to be a key molecule for the actin cytoskeletal reorganization regulated by Cdc42.

Acknowledgments—We thank the cDNA group of Kazusa DNA Research Institute for providing a cDNA of IQGAP1, Dr. I. Yahara (Tokyo Metropolitan Institute of Medical Science, Japan) for providing an anti-actin polyclonal antibody, and Dr. E. Nishida (Kyoto University, Japan) and Dr. M. Inagaki (Aichi Cancer Center Research Institute, Japan) for helpful discussion.

REFERENCES
1. Mackay, D., Nobs, C. D., and Hall, A. (1995) Trends Neurosci. 18, 496–501
2. Chant, J., and Stowers, L. (1995) Cell 81, 1–4
3. Konema, R., Ahmed, S., Best, A., and Polakis, P. (1995) Mol. Cell. Biol. 15, 1942–1952
4. Nobs, C. D., and Hall, A. (1995) Cell 81, 53–62
5. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992) Cell 70, 401–410
6. Manners, E., Leung, T., Sahibuddin, H., Zhao, Z. S., and Lim, L. (1994) Nature 367, 40–46
7. Manners, E., Cheng, C., Zhao, Z. S., Leung, T., Michael, G., Hall, C., and Lim, L. (1995) J. Biol. Chem. 270, 25070–25075
8. Martin, G. A., Bollag, G., McCormick, F., and Abo, A. (1995) EMBO J. 14, 1970–1978
9. Aspenstrom, P., Lindberg, U., and Hall, A. (1996) Curr. Biol. 6, 70–75
10. Symons, M., Derry, J. M. J., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Francke, U., and Abo, A. (1996) Cell 84, 723–734
11. Hart, M. J., Callow, M. G., Souza, B., and Polakis, P. (1996) EMBO J. 15, 2997–3005
12. McCallum, S. J., Wu, W. J., and Cerione, R. A. (1996) J. Biol. Chem. 271, 21752–21757
13. Brdić, S., Li, S., Lyman, C. W., Church, D. M., Wasmuth, J. J., Weissbach, L., Bernardas, A., and Snijders, A. J. (1996) Mol. Cell. Biol. 16, 4869–4878
14. Kuroda, S., Fukata, M., Kobayashi, K., Nakafuku, M., Nomura, N., Iwamatsu, A., and Kaibuchi, K. (1996) J. Biol. Chem. 271, 23363–23367
15. Zhang, S., Han, J., Sells, M. A., Chernoff, J., Knaus, U. G., Ulevitch, R. J., and Bokoch, G. M. (1995) J. Biol. Chem. 270, 23934–23936
16. Miki, H., Miura, K., and Takenawa, T. (1996) EMBO J. 15, 5326–5335
17. Castresana, J., and Saraste, M. (1995) FEBS Lett. 374, 149–151
18. Winder, S. J., and Walsh, M. P. (1990) J. Biol. Chem. 265, 10148–10155
19. Garlin, J. B., Yamin, R., Egan, S., Stewart, M., Stossel, T. P., Kwiatkowski, D. J., and Hartwig, J. H. (1990) J. Cell Biol. 111, 1109–1115

DISCUSSION

We have previously found that IQGAP1 accumulated at the insulin- and Rac1-induced membrane ruffling area in KB cells, where cortical actin filament was observed (14). In this study, we found that IQGAP1 directly interacts with F-actin and cross-links the actin filament. Cdc42 and Rac1 have been shown to regulate the filopodia and lamellipodia formation through the reorganization of actin filament meshwork (1, 2). Cross-linking of F-actin may be critical for the filopodia and lamellipodia formation. Our result strongly suggests that IQGAP11 plays an important role in these processes through the regulation of actin filament.

The target molecules for Cdc42 and Rac1 have thus far been identified to be PAK (6–8), WASP (9, 10), and IQGAP1 (11–14). Recently, it is reported that microinjection of activated PAK induces the filopodia formation and membrane ruffling in Swiss 3T3 cells (34). Another group reported that expression of constitutively activated PAK induces dynamic morphological changes (35). However, these results do not exclude the possibility that other targets are involved in the filopodia and lamellipodia formation. WASP (9, 10) and its isoform, N-WASP (16), are shown to alter the actin filament localization and to sever the F-actin, respectively. On the basis of these observations, it is plausible that IQGAP1, together with PAK and WASP, plays an important role in the reorganization of actin filament.

Actin-cross-linking proteins, such as α-actinin (20), filamin (22), and fimbrin (23) are believed to bridge F-actin and consequently cross-link the F-actin (25). Although IQGAP1 has a CHD in its amino terminus (aa 1–216) and this domain is sufficient for the interaction with F-actin, this domain lacked the F-actin-cross-linking activity. The IQGAP1-N (aa 1–863) had the F-actin binding and cross-linking activities. Additionally, oligomerization of IQGAP1 is mediated by its amino-terminal portion (aa 216–683). On the basis of these observations, it is likely that the CHD is sufficient for the binding to F-actin and the portion (aa 216–683) is responsible for the oligomerization of IQGAP1. Thus, IQGAP1 can cross-link the actin filament in a similar fashion to other actin-cross-linking proteins.
IQGAP1 and Actin

29583

20. Meyer, R. K., and Aebl, U. (1990) *J. Cell Biol.* **110**, 2013–2024

21. de Arruda, M. V., Watson, S., Lin, C. S., Leavitt, J., and Matsudaira, P. (1990) *J. Cell Biol.* **111**, 1069–1079

22. Nunnally, M. H., Powell, L. D., and Craig, S. W. (1981) *J. Biol. Chem.* **256**, 2083–2086

23. Bretscher, A. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78**, 6849–6853

24. Hartwig, J. H., and Kwiatkowski, D. J. (1991) *Curr. Opin. Cell Biol.* **3**, 87–97

25. Matsudaira, P. (1991) *Trends Biochem. Sci.* **16**, 87–92

26. Amano, M., Mukai, H., Ono, Y., Chihara, K., Matsu, T., Hamajima, Y., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) *Science* **271**, 648–650

27. Pardee, J. D., and Spudich, J. A. (1982) *Methods Enzymol.* **85**, 164–181

28. Hughes, C. A., and Bennett, V. (1995) *J. Biol. Chem.* **270**, 18990–18996

29. MacLean-Fletcher, S. D., and Pollard, T. D. (1980) *J. Cell Biol.* **85**, 414–428

30. Berryman, M., Gary, R., and Bretscher, A. (1995) *J. Cell Biol.* **131**, 1231–1242

31. Iwamatsu, A., and Yoshida-Kabomura, N. (1996) *J. Biochem. (Tokyo)* **120**, 29–34

32. Weissbach, L., Settleman, J., Kalady, M. F., Snijders, A. J., Murthy, A. E., Yan, Y. X., and Bernards, A. (1994) *J. Biol. Chem.* **269**, 20517–20521

33. Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M., and Stromer, M. H. (1976) *J. Biol. Chem.* **251**, 6860–6870

34. Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bekoch, G. M., and Chernoff, J. (1997) *Curr. Biol.* **7**, 202–210

35. Manser, E., Huang, H. Y., Loo, T. H., Chen, X. Q., Dong, J. M., Leung, T., and Lim, L. (1997) *Mol. Cell. Biol.* **17**, 1129–1143

36. Santos, E., Nebreda, A. R., Bryan, T., and Rempner, E. S. (1988) *J. Biol. Chem.* **263**, 9853–9858

37. Mizuno, T., Kaibuchi, K., Yamamoto, T., Kawamura, M., Sakoda, T., Fujioka, H., Matsuura, Y., and Takai, Y. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 6442–6446

38. Bashour, A. M., Fullerton, A. T., Hart, M. J., and Bloom, G. S. (1997) *J. Cell Biol.* **137**, 1555–1566