The Zinc- and Calcium-binding S100B Interacts and Co-localizes with IQGAP1 during Dynamic Rearrangement of Cell Membranes*

Received for publication, May 30, 2002, and in revised form, October 8, 2002
Published, JBC Papers in Press, October 10, 2002, DOI 10.1074/jbc.M205363200

Gaelh Ouengue Mbele‡, Jean Christophe Deloulme‡, Benoît Jean Gentil‡, Christian Delphin‡, Myriam Ferro§, Jérôme Garin§, Miyoko Takahashi¶, and Jacques Baudier†

From the ‡Département Réponse et Différenciation Cellulaires du Commissariat à l’Energie Atomique (CEA), INSERM U-014 DRDC-TS, §Laboratoire de Chimie des Protéines DRDC-CP, CEA, Grenoble 38054, France and ¶Syn-X Pharma, Inc., 6554 Visaount Rd., Mississauga, Ontario L4V 1H4, Canada

The Zn2+- and Ca2+-binding S100B protein is implicated in multiple intracellular and extracellular regulatory events. In glial cells, a relationship exists between cytoplasmic S100B accumulation and cell morphological changes. We have identified the IQGAP1 protein as the major cytoplasmic S100B target protein in different rat and human glial cell lines in the presence of Zn2+ and Ca2+. Zn2+ binding to S100B is sufficient to promote interaction with IQGAP1. IQ motifs on IQGAP1 represent the minimal interaction sites for S100B. We also provide evidence that, in human astrocytoma cell lines, S100B co-localizes with IQGAP1 at the polarized leading edge and areas of membrane ruffling and that both proteins relocate in a Ca2+-dependent manner within newly formed vesicle-like structures. Our data identify IQGAP1 as a potential target protein of S100B during processes of dynamic rearrangement of cell membrane morphology. They also reveal an additional cellular function for IQGAP1 associated with Zn2+/Ca2+-dependent relocation of S100B.

S100B is a member of the S100 family of proteins containing two EF-hand-type calcium-binding domains (1). This protein interacts not only with Ca2+ but also with Zn2+ ions, binding Zn2+ ions with an affinity in the nanomolar range (2). The capacity of S100B to bind and release Zn2+ suggests that Zn2+ may not only play a structural role but might also be involved, together with Ca2+, in concerted regulation of S100B function. The S100B protein is naturally highly expressed in the vertebrate nervous system, where it is present in astrocytes and Schwann cells (3). In the adult central nervous system, the S100B protein is present in the nuclei and cytoplasm of astrocytes and accumulates in the astrocytic dendrites in the perivascular processes (4). Studies in different laboratories suggest a variety of intracellular regulations by S100B, including negative cell growth regulation (5), cell structure (6), and calcium homeostasis (7). The S100B protein is also secreted from astrocytes and has extracellular functions (8). Extracellular S100B acts as a modulator of neuronal synaptic plasticity (9). Although nanomolar quantities have beneficial neurotrophic effects on nerve cells, high levels of this protein have been implicated in glia activation and could contribute to the development of brain pathology as observed in Down’s syndrome and Alzheimer’s disease (10). The recent observation that S100B triggers activation of the pro-inflammatory cell surface receptor for advanced glycation end products has shed more light on its extracellular function (11). In cultured human astrocytoma U87 cells, S100B secretion is dependent on relocation of S100B toward vesicle-like structures at the periphery of the cells and is regulated by Ca2+ and Zn2+ (12). S100B can also be secreted into the bloodstream and cerebrospinal fluid and is a biochemical marker of brain damage or dysfunction in acute and chronic diseases (13, 14). A relationship between S100B accumulation in the astrocytic end-feet and morphological changes of astrocytes in the perivascular regions has been reported previously (15). These changes may be related to the release of S100B into the blood stream (15). Consistent with dynamic regulation of astrocyte cell shape by S100B, antisense inhibition of S100B production in cultured rat glial C6 cells is correlated with alterations in cellular morphology (6). The mechanisms of regulation of astrocyte cell morphology by S100B and its secretion pathway remain unclear. By analogy with other EF-hand Ca2+-binding proteins, such as calmodulin, one might suppose that the biological activity of S100B is related to Ca2+/Zn2+-dependent interaction with target proteins. In this study, we identify IQGAP1 protein as the first S100B target protein identified to date whose interaction with S100B is regulated by Zn2+ and Ca2+. IQGAP1 is also the major specific cytoplasmic S100B target protein present in both rat glial C6 and human U373 or U87 astrocytoma cell lines. We also provide evidence that cytoplasmic S100B specifically binds to a sub-population of IQGAP1 molecules that localize at the polarized leading edge and areas of membrane ruffling and that both S100B and IQGAP1 proteins are relocated in a Ca2+-dependent manner within vesicle-like structures. The interaction of S100B with IQGAP1 may have important implications for understanding the roles played by S100B in processes of dynamic rearrangement of cell membranes and in the mechanisms of Zn2+/Ca2+-dependent relocation and secretion of S100B.

MATERIALS AND METHODS

Cell Cultures and 35S Met/Cys Labeling—Human astrocytoma U-373MG, U-87MG cells, rat gloma C6 cells, mammary carcinoma MCF7 cells, and NIH 3T3 fibroblasts were cultured in Dulbecco’s modified Eagle’s medium with Glutamax (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen). Cells were labeled in methionine-free minimal essential medium, 5% fetal calf serum supplemented with 35S Met/Cys mix (50 μCi/ml) for 6 h.

Transfection Experiments—U-373MG, U-87MG, NIH 3T3, and MCF7 cells were transfected with the pcDNA-Neo containing the wild-type or C-terminal deleted S100B cDNA (17) using FuGENETM 6 reagent transfection according to manufacturer’s protocol. For stably transfected S100B-MCF7 cell lines, cells were incubated, 48 h after

* This work was supported by grants from the Association pour la Recherche sur le Cancer and la Ligue Nationale Contre le Cancer. The authors thanks Dr. Jerome Garin for plasmid construction and Dr. Myriam Ferro for helpful discussions.

‡ To whom correspondence should be addressed. Tel.: 33-438-78-43-28; Fax: 33-438-78-58-89; E-mail: jbaudier@cea.fr.

This paper is available on line at http://www.jbc.org
transfection, in complete medium supplemented with 500 μg/ml -lactam, and neomycin-resistant S100B-MCF7 clones were

**Primary Antibodies**—Monoclonal anti-β-tubulin antibody was a gift from Drs. L. Paturle and D. Job (Laboratoire du cytosquelette, CEN-Grenoble). Polyclonal rabbit anti-S100B antibodies (20311 and A5110) were from Dako. Purified S100B monoclonal antibody S16 was previously described (18). Monoclonal anti-calmodulin (C-7055) and monoclonal anti-S100A6 antibody (S3046) were from Sigma. The monoclonal mouse anti-caldesmon (05-173) and the monoclonal mouse anti-IQGAP1 AF4 (05-504) antibodies were from Upstate Biotechnology. IQGAP1 AF4 antibody was used for immunoprecipitation experiments. The mouse monoclonal anti-IQGAP1 (mAb IgG1, I53820) antibody used at 1:2000 in Western blot analyses. The mouse monoclonal anti-IQGAP1 (mAb IgG1, I53820) antibody was from Transduction Laboratories (C19220). Polyclonal rabbit anti-Cdc42 (Erickson (Cornell University, Ithaca, NY) and was used at 1:2000 for

**RESULTS**

- The abbreviations used are: CaM, calmodulin; Tricine, N-2-hydroxy-1,1-bis(hydroxymethyl)glycine; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MS/MS, tandem mass spectrometry; GTP-S, guanosine 5’-3-O-(thio)triphosphate; GFP, green fluorescent protein.

**Interaction of S100B Protein with IQGAP1**

1 The abbreviations used are: CaM, calmodulin; Tricine, N-2-hydroxy-1,1-bis(hydroxymethyl)glycine; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MS/MS, tandem mass spectrometry; GTP-S, guanosine 5’-3-O-(thio)triphosphate; GFP, green fluorescent protein.
Although all cells are uniformly immunostained with S100A6 antibodies (Fig. 2B), considerable variation in S100B immunostaining characterized post-confluent U373 cells. In post-confluent culture, the strongest S100B-positive cells had grown on top of the cell layer. These cells are characterized by intense cytoplasmic S100B immunoreactivity and have adopted a less flattened morphology with long processes. Confocal microscopy analysis of S100B immunostaining in confluent and post-confluent U373 cells confirmed a relationship between S100B overexpression and change in cell shape (Fig. 2, C and D). In these experiments, cells were double-labeled with S100B polyclonal antibodies (red) and IQGAP1 monoclonal antibody (green). U373 cells that enter confluence have a flattened morphology. In these cells, the weak S100B immunoreactivity is mostly nuclear and IQGAP1 accumulates at the cell periphery (Fig. 2C). In post-confluent culture, cells characterized by intense cytoplasmic S100B immunoreactivity have adopted a less flattened morphology with long processes (Fig. 2D). Overlapping of the S100B and IQGAP1 stainings (white pixels) reveals that some of the S100B colocalizes with IQGAP1 at the cytoplasmic membrane and within processes. The correlation between cytoplasmic S100B overexpression with changes in cell shape is consistent with previous studies that showed that selective inhibition of S100B production by antisense strategies in rat glioma C6 cells resulted in a more flattened cellular morphology (6). In rat C6 glioma cells, S100B is also up-regulated in post-confluent cells, and its up-regulation correlates with drastic cell morphological changes (data not shown).

IQGAP1 Is the Major Specific S100B Binding Protein in the Gliial C6 and U373 Cells—In an attempt to identify specific S100B target proteins that could mediate the effect of S100B on cell morphology, we compared proteins in astrocytoma U373 and glial C6 cell extracts that bind to S100B-Sepharose beads. A major S100B-binding protein that migrated with an apparent molecular mass of 170 kDa was identified in both cell lines (Fig. 3A). The 170-kDa protein binds to S100B-Sepharose beads in EGTA/EDTA- and Ca\(^{2+}/\)Zn\(^{2+}\)-containing buffer. The 170-kDa human protein from U373 MG cells was further characterized by mass spectrometry. Protein identification was achieved using both MALDI peptide mass fingerprints and MS/MS sequence information (see “Materials and Methods”). Results revealed that it corresponds to human IQGAP1. IQ-GAP1 is a widely expressed protein that acts as a scaffold in recruiting and maintaining the organization of cytoskeletal proteins at the plasma membrane (19–27). The other high molecular weight Ca\(^{2+}/\)Zn\(^{2+}\)-dependent S100B-binding protein present in glial C6 cells, but not in U373 cell extract, has been previously identified as AHNAK (28). The binding of IQGAP1 to S100B is specific, because it is not observed with S100A6 (Fig. 3B, lanes 5 and 6), and S100A11 (Fig. 3C, lanes 3 and 7), two other S100 species expressed in U373 cells (17). S100A1, the closest S100B homologue that is not expressed in U373 cells (17), also binds IQGAP1 (Fig. 3B, lanes 3 and 4). IQGAP1 in U373 cell extract also binds to calmodulin-Sepharose beads (Fig. 3C, lanes 4 and 8).

IQGAP1 Co-immunoprecipitates with S100B from U373 Cell Extract—A physical interaction between S100B with IQGAP1 was confirmed by co-immunoprecipitation of a S100B/IQGAP1 complex from confluent cell extract (Fig. 4). In a first set of experiments, S100B was immunoprecipitated with S16 monoclonal S100B antibody that recognizes an epitope located within the N terminus of S100B (18). The presence of IQGAP1 in the S100B immunoprecipitate was revealed with anti-IQGAP1 polyclonal antibodies. A small but detectable amount of IQGAP1 is found in the S100B immunoprecipitates in EDTA/EGTA buffer (Fig. 4A, lane 4). The amount of IQGAP1 immunoprecipitated with S100B monoclonal antibody increased substantially in buffer containing Ca\(^{2+}/\)Zn\(^{2+}\) (Fig. 4A, lane 5). The co-immunoprecipitation of IQGAP1 with S100B is specific, because it is not observed with control anti-MyoD antibodies (Fig. 4A, lanes 2 and 3). The Ca\(^{2+}/\)Zn\(^{2+}\) requirement for the interaction between soluble S100B and IQGAP1 contrasts with the apparent divalent ion-independent interaction observed with S100B cross-linked to Sepharose beads (see below).

In a second set of experiments, IQGAP1 was immunoprecipitated with anti-IQGAP1 AF4 monoclonal antibody (Fig. 4B). S100B is found associated with IQGAP1 immunoprecipitates when using Ca\(^{2+}/\)Zn\(^{2+}\)-containing buffer. Cdc42 and \(\beta\)-catenin, two other IQGAP1 target proteins (22–24) are also found associated with IQGAP1 immunoprecipitates in both EGTA/EDTA and Ca\(^{2+}/\)Zn\(^{2+}\) buffer. Several laboratories have also shown intracellular interactions between CaM and IQGAP1 (19–20, 26, 29). In Fig. 4C, we compared the association of calmodulin (CaM) and S100B with immunoprecipitate IQGAP1 out of exponentially growing and post-confluent U373 cells in EGTA/EDTA- and Ca\(^{2+}/\)Zn\(^{2+}\)-containing buffer. CaM and S100B that co-immunoprecipitated with IQGAP1 were sequentially revealed using the same nitrocellulose transfer membrane. CaM co-immunoprecipitates with IQGAP1 in all conditions tested, whereas S100B only co-immunoprecipitates with IQGAP1 from post-confluent cell extracts in buffer containing Ca\(^{2+}/\)Zn\(^{2+}\) (compare lanes 2 and 3). It is noteworthy that, although other laboratories reported that Ca\(^{2+}\) enhances the interaction between CaM and IQGAP1 (26, 29–30), we found more CaM immunoreactivity associated with IQGAP1 in U373 cell extracts containing EGTA and EDTA (compare lanes 2 and 3 or lanes 5 and 6). This unexpected observation cannot solely be explained by a competition with S100B, because it is also observed with sub-confluent culture characterized by low S100B expression.

Zn\(^{2+}\)-dependent Interaction between S100B and IQGAP1—In a pull-down assay using S100B cross-linked onto Sepharose beads, the S100B/IQGAP1 interaction can be detected independently of the presence of EGTA/EDTA or Ca\(^{2+}/\)Zn\(^{2+}\) in binding buffer (Fig. 3). In contrast, co-immunoprecipitation experiments with endogenous cellular proteins revealed that IQGAP1/S100B interaction is markedly strengthened when Ca\(^{2+}\) and Zn\(^{2+}\) are included in binding buffer (Fig. 4).
One factor that might explain this apparent discrepancy is the high S100B protein concentration used in the pull-down assay (1.5–3 μM) compared with the soluble S100B in cell extracts. To evaluate the effect of S100B concentration on complex formation with IQGAP1, we performed co-immunoprecipitation analysis using MCF7 cells extracts (which do not express the S100B protein) supplemented with increasing concentrations of recombinant human S100B (Fig. 5A). Results confirm that the S100B/IQGAP1 interaction is regulated by divalent ions and that high concentrations of S100B are not sufficient to promote ion-independent interactions.

Previous studies from our laboratory have shown that chemical modifications within the S100B molecule may have profound effects on the protein quaternary and tertiary structures (2, 31). We thus investigated the possibility that cross-linking of S100B onto Sepharose beads modifies S100B conformation as to favor interaction with IQGAP1. To test this, we compared the interaction of recombinant IQGAP1 produced in rabbit reticulocytes with the equal amount of soluble S100B, by means of co-immunoprecipitation, and S100B cross-linked onto Sepharose beads by pull-down assays. As shown in Fig. 5B, S100B cross-linked onto Sepharose beads, but not soluble S100B, interacts with in vitro translated IQGAP1 in EGTA/EDTA-containing buffer (compare lanes 3 and 7). We next evaluated the contribution of individual divalent ions, Zn$^{2+}$ and Ca$^{2+}$, to the S100B/IQGAP1 interaction. In the co-immunoprecipitation and pull-down assays, addition of Zn$^{2+}$ (10 μM) to binding buffer stimulates interaction between S100B and IQGAP1 (lanes 4 and 8). Further addition of Ca$^{2+}$ repeatedly enhanced that interaction (lanes 5 and 9). Quantitative evaluation of the radioactivity associated with S100B-Sepharose beads in different buffer conditions is shown in Fig. 5C. Together, these data suggest that, when cross-linked onto Sepharose beads, S100B adopts a conformation that favors its interaction with IQGAP1. This conformational state is probably very similar to that induced upon Zn$^{2+}$ binding. They also suggest that Ca$^{2+}$ might also strengthen the S100B/IQGAP1 interaction.

Zn$^{2+}$-dependent interaction of S100B with IQGAP1 was also observed with cellular proteins (Fig. 5D). NIH-3T3 cells were
transfected with S100B expression plasmid and S100B/IQGAP1 complex formation analyzed by co-immunoprecipitation with S16 monocalonal antibody in different buffer conditions. When transfected cells are lysed in binding buffer containing 20 μM EGTA, the S100B/IQGAP1 interaction is almost undetectable (lane 1). If cell extract containing 20 μM EGTA is supplemented with Zn2+ (40 μM), binding of IQGAP1 to S100B is rescued (lane 2). Addition of Ca2+ (300 μM) or Zn2+ plus Ca2+ to EGTA containing cell extract also rescues S100B/IQGAP1 interaction (lanes 3 and 4). As observed in *in vitro* translated 35S-labeled IQGAP1 (Fig. 5, B and C), a slight but significant increase in IQGAP1 immunoreactivity is found associated with S100B immunoprecipitates in buffer containing Ca2+. That stimulation was more clearly seen with lower exposure of the Western blot membrane to ECL film. All together these data suggest that, in solution, the S100B/IQGAP1 interaction requires either Zn2+ or Ca2+ ions and that Zn2+ is sufficient to promote that interaction. IQGAP1 is thus the first S100B target protein identified whose interaction with S100B is mediated by Zn2+-dependent conformational change on S100B.

**Mechanism of Zn2+-dependent Interaction of S100B with IQGAP1**—To further confirm the essential role of Zn2+-dependent conformational change on S100B for interaction with IQGAP1, we next compared the mechanism of S100B with IQGAP1 and with a strict calcium-dependent target protein, AHNAK (28). We first studied the interactions of the wild-type S100B and of a C-terminal deleted mutant S100B (S100BΔCt) with IQGAP1 and AHNAK. We used S100BΔCt because the C terminus domain of S100B is required for interactions between S100B and strict Ca2+-dependent target protein (32, 33). NIH 3T3 cells were transfected with expression vectors encoding S100B or S100BΔCt, and complex formation was assayed by co-immunoprecipitation using the N-terminal S100B monoclonal antibody S16 (Fig. 6A). Although we repeatedly observed a much lower expression of S100BΔCt compared with wild-type S100B, both wild-type S100B and mutant S100BΔCt co-immunoprecipitate with IQGAP1 from cell lysates in Zn2+/Ca2+-containing buffer (lanes 6 and 9). Deletion of the C terminus of S100B specifically abrogated Zn2+/Ca2+-dependent interaction of S100B with AHNAK (compare lanes 6 and 9). These results suggest that the C terminus of S100B is not implicated in Zn2+-dependent interaction of S100B with IQGAP1. We next compared the contribution of individual divalent ions, Zn2+ and Ca2+, to the S100BΔCt/IQGAP1 interaction (Fig. 6B). When transfected NIH 3T3 cells are lysed in binding buffer containing 20 μM EGTA, the S100BΔCt/IQGAP1 interaction is almost undetectable (lane 2). If cell extract containing 20 μM EGTA is supplemented with Zn2+ (40 μM) (lane 3) or Zn2+ plus Ca2+ (lane 5), binding of IQGAP1 to S100BΔCt is rescued. However, in contrast to the full-length S100B (Fig. 5D, lane 3), addition of Ca2+ (300 μM) alone also stimulates S100BΔCt/IQGAP1 interaction, but to a much lower extent than Zn2+ or Zn2+ plus Ca2+ (Fig. 6B, lane 4). All together these data confirm that, in solution, Zn2+ is sufficient to promote the S100B/IQGAP1 interaction and that Ca2+ binding to S100B might contribute to strengthen the interaction via the C terminus of S100B.

**Mapping the Minimal Interaction Domain for S100B on IQGAP1**—To investigate which domains of IQGAP1 are responsible for S100B binding, the full-length protein and the indicated mutants of IQGAP1 were produced in rabbit reticulocytes (Fig. 7A), and their interaction with S100B- and CaM-Sepharose beads compared (Fig. 7B), as described under “Materials and Methods.” There is no difference between the full-length IQGAP1 and the N-terminal domain of IQGAP1 in binding S100B and CaM beads. All fusion proteins containing the IQ domain (IQ, CHD-IQ, and IR-IQ) also bind to S100B and CaM in the presence of EGTA/EDTA. These findings suggest that IQ motifs are essential for interactions between S100B and IQGAP1. They are also consistent with previous data showing that the high affinity CaM binding region on IQGAP1 corresponds to its IQ domains (29).

To confirm that S100B and the CaM-binding domain on IQGAP1 overlap and to investigate if S100B competes with
Interaction of S100B Protein with IQGAP1

---

**Fig. 6.** Mechanism of Zn\(^{2+}\)-dependent interaction of S100B with IQGAP1. A, NIH 3T3 fibroblasts were transfected with empty (control) plasmid (lanes 1–3), or with wild-type (wt) S100B (lanes 4–6) or mutant (ΔCt) S100BΔCt (lanes 7–9) plasmids for 36 h by the calcium phosphate method. Cell extracts were immunoprecipitated using the monoclonal anti-S100B S16 antibody (lanes 2–3, 5–6, and 8–9). Immunoprecipitations were performed in the absence (lanes 2, 5, and 8) or in the presence (lanes 3, 6, and 9) of Ca\(^{2+}/Zn\(^{2+}\). Lane 1, 4, and 7 are total cell extracts used for immunoprecipitation. B, NIH 3T3 cells were transfected with control plasmid (lane 1) or with S100BΔCt plasmid (lanes 2–5) as in A. S100BΔCt was immunoprecipitated with monoclonal anti-S100B S16 antibody in buffer containing 20 μg EGTA, 20 μg EGTA plus 40 μg ZnSO\(_4\) (lane 3), 20 μg EGTA plus 0.3 mM CaCl\(_2\) (lane 4), or 20 μg EGTA plus 40 μg ZnSO\(_4\) and 0.3 mM CaCl\(_2\) (lane 5). In A and B, protein complexes were resolved by SDS-PAGE, transferred to nitrocellulose membrane, and probed with monoclonal anti-IQGAP1, polyclonal anti-AHNAK, and polyclonal rabbit anti-S100B. Immune complexes were visualized by an ECL kit.

---

**Fig. 5.** Zn\(^{2+}\)-dependent interaction between S100B and IQGAP1. A, MCF7 cell extracts in buffer containing 5 mM EDTA and 5 mM EGTA (lanes 2–6) or 0.3 mM CaCl\(_2\) and 10 μM ZnSO\(_4\) (lanes 7–11) were not supplemented (lanes 2–3 and 7–8) or supplemented with purified human recombinant S100B at concentrations of 1 μM (lanes 4 and 9), 2 μM (lanes 5 and 10), and 4 μM (lanes 6–11) prior to immunoprecipitation with control anti MyoD antibody (lanes 2 and 7) or monoclonal anti-IQGAP1 AP4 antibody (lanes 3–6 and 8–11). Lane 1 is total cell extract used for immunoprecipitation. Protein complexes were resolved by SDS-PAGE, transferred to nitrocellulose membranes, and probed with monoclonal anti-IQGAP1 or with polyclonal anti-S100B antibodies. Immune complexes were visualized using an ECL kit. B, [\(^{35}\)S]methionine-labeled recombinant IQGAP1 produced in rabbit reticulocyte was mixed with soluble purified recombinant S100B (2 μM), then S100B was immunoprecipitated with monoclonal anti-S100B S16 antibody (1 μg) (lanes 3–5) or with S100B-Sepharose beads (2 μM) (lanes 7–9) in buffer containing 5 mM EDTA and 5 mM EGTA (lanes 3 and 7), 10 μM ZnSO\(_4\) (lanes 4 and 8), or 0.3 mM CaCl\(_2\) and 10 μM ZnSO\(_4\) (lanes 5 and 9). Protein complexes were resolved by SDS-PAGE and autoradiography. Lanes 1 and 6 correspond to total reticulocyte lysate. Lane 2 is immunoprecipitation with control anti MyoD antibody. C, quantitative analysis of the radioactivity associated with S100B-Sepharose beads. The amount of IQGAP1 bound to calmodulin, in the presence of various concentrations of divalent ions and of S100B, was quantified by measuring the radioactivity associated with the CaM-beads pellet (Fig. 7C). In the absence of divalent ion, S100B interfered with the association of IQGAP1 with CaM only at high concentration. In the presence of Zn\(^{2+}\), or Zn\(^{2+}\) plus Ca\(^{2+}\), S100B produced a dose-dependent inhibition of binding of IQGAP1 to CaM. The S100B concentration-dependent inhibition curves show that, in the presence of Zn\(^{2+}\), or Zn\(^{2+}\) plus Ca\(^{2+}\), half inhibition occurs with a S100B concentration below the estimated CaM-Sepharose concentration in the assay (1.8 μM), suggesting that, in its Zn\(^{2+}\)- or Ca\(^{2+}/Zn\(^{2+}\)-bound conformations S100B may have higher affinity for IQGAP1 than CaM. As expected, when purified bovine brain CaM was used in competition with CaM-Sepharose, in the presence of Ca\(^{2+}\) and Zn\(^{2+}\), for binding IQGAP1, the inhibition curve is shifted to a higher competitor protein concentration (Fig. 7C). Inversely, when the purified competitor proteins, CaM and S100B, were mixed with \(^{35}\)S-labeled recombinant IQGAP1 together with S100B-Sepharose beads, CaM antagonized IQGAP1 binding to S100B-Sepharose at a much higher concentration than S100B (Fig. 7D) confirming that in its Ca\(^{2+}/Zn\(^{2+}\)-bound conformations S100B has a higher affinity for in vitro translated IQGAP1 than has CaM.

S100B Co-localizes with IQGAP1 at Ruffling Membranes but Not at Sites of Cell-Cell Contact in U373 Cells—The specificity of interaction between S100B and IQGAP1 in U373 cells was then investigated at the cellular level by indirect double im-
IQGAP1 may associate with Cdc42 or IQGAP1 could be implicated in the S100B secretion pathway (12). To evaluate whether IQGAP1 could be implicated in the S100B secretion pathway, we studied the effect of intracellular Ca²⁺ increase on S100B and IQGAP1 localization in U87 cells. U87 cells were transiently transfected with the S100B expression plasmid and fixed. S100B and IQGAP1 localization were analyzed by immunocytochemistry (Fig. 9). In U87 cells grown in normal medium, ectopically expressed S100B protein localizes...
to membrane ruffling, the perinuclear area, and within the cell nuclei (Fig. 9A). Ionomycin stimulation of U87 cells induces rapid relocation of S100B within vesicle-like structures that have the appearance of membrane blebs (Fig. 9B). As previously noticed (12), these vesicle-like structures are often located toward the periphery of the cells. When considering IQGAP1 in non-stimulated U87 cells, IQGAP1 is diffusely located within the cytoplasm and accumulates at membrane ruffling (Fig. 9A). Increase in the intracellular Ca²⁺ concentration causes relocation of IQGAP1 to the newly formed membrane blebs where it co-localizes with S100B (Fig. 9B). IQGAP1 relocation did not depend on the expression levels of S100B, because it is also observed in non-transfected cells.

**Ca²⁺ Regulates Both Relocation and Stable Association of S100B and IQGAP1 with Membrane Vesicles in MCF7 Cells**—As observed in most epithelial cells, in MCF7 human breast epithelial cells, IQGAP1 accumulates at the cell-cell junction (Ref. 24 and Fig. 10A). With MCF7 cells stably transfected with the S100B gene (S100B-MCF7) and grown in complete culture medium, IQGAP1 remains concentrated at the cell-cell junction, whereas the ectopically expressed S100B protein accumulates within the cell nuclei (Fig. 10A). When S100B-MCF7 cells are cultured in serum-free medium, intracellular calcium elevation mediated by calcium ionophore ionomycin produced a rapid relocation of both S100B and IQGAP1 within the cell cytoplasm (Fig. 10B). When ionomycin-containing medium was replaced by fresh complete medium, both S100B and IQGAP1 relocated to the cell nuclei and the cell-cell junctions, respectively, indicating that Ca²⁺-dependent translocation is reversible. However, in many S100B-MCF7 cells, a co-localization of S100B and IQGAP1 persisted within newly formed membrane blebs located toward the periphery of the cells (Fig. 10C).

**DISCUSSION**

**Characterization of the Molecular Interaction between S100B and IQGAP1**—In solution, S100B associates as a non-covalent dimer. In its dimeric form, S100B interacts not only with Ca²⁺ but also with Zn²⁺ ions (2). S100B binds Zn²⁺ ions with affinity in the nanomolar range (2). In contrast, the S100B dimer affinity for calcium is rather weak compared with other EF-hand calcium-binding proteins and is not within the range of physiological intracellular calcium concentrations (2). In the presence of Zn²⁺, or upon alkylation of Zn²⁺ ligand Cys84, the S100B adopts a “Ca²⁺-bound-like” conformation (2, 31). That conformation is associated with destabilization of the quaternary protein structure, exposure of the calcium-binding sites to solvent, and increased apparent Ca²⁺ affinity compatible with local intracellular calcium concentration (2, 31). The capacity of S100B to bind and release Zn²⁺ without denaturation suggests that Zn²⁺ may not only play a structural role but might be involved, together with Ca²⁺, in concerted regulation of S100B interaction with target proteins. In this study, we identified for the first time a Zn²⁺-dependent S100B target protein, IQGAP1. Zn²⁺-bound S100B co-immunoprecipitates with IQGAP1 present in cell extract or expressed in rabbit reticulocyte. In contrast to immunoprecipitation assay, pull-down assay using...
S100B cross-linked onto Sepharose beads revealed that the S100B/IQGAP1 interaction can also be detected in the presence of EGTA/EDTA in binding buffer. We have shown that these discrepancies probably result from differences in conformation between the soluble S100B and the S100B cross-linked onto Sepharose beads (Fig. 5B). This was further confirmed by competition experiments using CaM-Sepharose. Only in its Zn\(^{2+}\)- or Zn\(^{2+}\)/Ca\(^{2+}\)-bound conformations is S100B capable of substantially antagonizing the binding of recombinant IQGAP1 to CaM-Sepharose beads (Fig. 7C). The mainly CaM-binding domains on IQGAP1 correspond to IQGAP1-IQ motifs (29). It is therefore likely that Zn\(^{2+}\)-dependent interactions of S100B with IQGAP1 also require IQGAP1-IQ motifs. This was confirmed by mapping the Ca\(^{2+}\)-independent S100B-binding domain on IQGAP1 using a pull-down assay (Fig. 7, A and B). The IQ motif was initially identified in brain-specific protein kinase C substrates neuromodulin (GAP43) and neurogranin as part of Ca\(^{2+}\)-independent CaM binding and protein kinase C phosphorylation site domain (35, 36). IQ motifs have since been identified as binding sites for CaM in a variety of proteins (37). We provide here evidence that the IQ motifs are not specific to CaM and can also be targeted by Zn\(^{2+}\)-bound S100B. The Zn\(^{2+}\)-dependent interaction of S100B with IQGAP1 is unique among the S100B-target proteins so far identified. With conventional S100B target proteins, Zn\(^{2+}\) does not promote direct interaction but modulate S100B Ca\(^{2+}\) affinity (28). Although Zn\(^{2+}\) binding to S100B is sufficient to promote interaction of S100B with IQGAP1, quantitative comparison of binding of full length recombinant IQGAP1 to S100B revealed that addition of Ca\(^{2+}\) to the binding buffer significantly potentiates the interaction between the two proteins (Fig 5B). A similar effect of Ca\(^{2+}\) was reported for CaM/IQGAP1 interaction (26, 30). It has been proposed that differences in CaM binding to IQGAP1 in the absence and in the presence of Ca\(^{2+}\) is attributable to difference between IQ motifs, some of which bind Ca\(^{2+}\)-CaM, and others bind Ca\(^{2+}\)-free CaM (26, 30). Because Zn\(^{2+}\)-bound S100B prevents the binding of IQGAP1 to Ca\(^{2+}\)-free CaM, it is possible that, in its Zn\(^{2+}\)-bound conformation, S100B interacts with some of the IQ motifs that are capable of binding CaM in the absence of Ca\(^{2+}\). When complexed to Ca\(^{2+}\), the S100B would then be able to utilize the four IQ motifs to further strengthen its interaction with IQGAP1. It is also possible that other domains on IQGAP1 could confer Ca\(^{2+}\) sensitivity to the S100B/IQGAP1 interaction. A striking amino acid sequence conservation exists between the IQGAP1–5 repeat motif (545INEALDEGDAQ550) and the Ca\(^{2+}\)-dependent S100B-binding domain on p53 (544INEALELKDAQ553) (38). The S100B-binding domain present on p53 is a tetramerization domain that is also implicated in the interaction of p53 with other regulatory proteins (39). Further studies should explore if the interaction of S100B with IQGAP1 could regulate IQGAP1 interactions with partner proteins through its repeat motifs.

IQGAP1 as a Mediator of S100B-induced Regulation of Cell Shape and S100B Secretion Pathway—In post-confluent human glioma U373 MG, the strongest S100B immunoreactivity is found associated with cells that are characterized by a less flattened morphology and long processes (Fig. 2). In these cells, a co-localization of S100B and IQGAP1 is evident at plasma membrane and within growing processes. Transient transfection of S100B in sub-confluent U373 MG cells confirmed that cytoplasmic S100B specifically co-localizes with IQGAP1 at the plasma membrane but not at sites of cell-cell junction (Fig. 8). Many studies have implicated IQGAP1 as a scaffold to recruit and localize protein complexes involved in actin and microtubule-based cellular functions at the plasma membrane (19, 20, 27, 34). Interaction of Ca\(^{2+}\)/Zn\(^{2+}\)-S100B with IQGAP1 could, therefore, regulate IQGAP1 scaffold function at the plasma membrane in response to incoming signals linked to the reorganization of the actin and microtubule cytoskeleton. This hypothesis is consistent with previous studies showing that selective inhibition of S100B expression by antisense strategies in rat glioma C6 cells resulted in a more flattened cellular morphology and a more organized actin stress fiber staining pattern with less membrane ruffling (6). It is noteworthy that IQGAP1 can also bind to S100A1 (Fig. 3B). S100A1, the closest S100B homologue, is also a potential regulator of cell cytoskeleton and cell morphology (41).

In this study, we have also provided evidence that the interaction between S100B and IQGAP1 might also occur within vesicle-like structures that also have the appearance of membrane blebs in the human astrocytoma U87 and MCF7 cells. In U87 cells, both S100B and IQGAP1 translocate and co-localize within vesicle-like structures in response to increased intracellular calcium. The Ca\(^{2+}\)-dependent translocation of S100B in U87 has been studied in detail elsewhere (12). It has been proposed that these vesicle-like structures, which correspond to cytoplasmic extensions that form and disappear dynamically, are implicated in Ca\(^{2+}\)-dependent S100B secretion process (12). In U87 cells not stimulated with ionomycin, these vesicle-like structures were rarely immunostained with IQGAP1 antibodies, suggesting that Ca\(^{2+}\) regulates targeting of IQGAP1 to these structures. The mechanism that controls Ca\(^{2+}\)-dependent IQGAP1 relocation is independent of S100B, because it is also observed in cells that do not express S100B. Ca\(^{2+}\)-dependent translocation of S100B and IQGAP1 within structures having the appearance of membrane blebs was confirmed with stably transfected S100B-MCF7 cells (Fig. 10). In stably transfected S100B-MCF7, IQGAP1 is naturally targeted to the cell-cell junctions and S100B accumulates predominantly within the cell nuclei. Serum deprivation coupled to intracellular calcium elevation mediated by ionomycin induces a relocation of both S100B and IQGAP1 within the cell cytoplasm. This process is maximal within 5 min, supporting the idea that it depends on calcium fluxes. When ionomycin-containing medium is replaced by fresh complete medium, the majority of S100B and IQGAP1 has a tendency to relocated to nuclei and to cell-cell junctions, respectively, indicating that Ca\(^{2+}\)-dependent translocation is reversible. However, in many S100B-MCF7 cells, a co-localization of S100B and IQGAP1 persisted within membrane blebs located toward the periphery of the cells. Hence, Ca\(^{2+}\) is required for both S100B and IQGAP1 relocation and their subsequent stable association with newly formed membrane structures. Taking into account the high affinity interaction between S100B and IQGAP1, we propose that IQGAP1 might be implicated in recruiting S100B to secretary vesicular structures and might be involved in the Ca\(^{2+}\)-dependent S100B secretion pathway. The observed Ca\(^{2+}\)-dependent association of IQGAP1 with vesicle-like structure is new and might reveal an additional cellular function for the protein. Whether or not IQGAP1 directly participates in stabilization, turnover, and dynamics of these vesicle-like structures has yet to be evaluated. It is significant that functional disruption of Ig1p, a yeast homologue of the mammalian IQGAPs, has been directly implicated in vesicle accumulation at the growing bud, suggesting a possible involvement of yeast Ig1p in secretion or some aspect of vesicle trafficking (40).

The interaction between cytoplasmic S100B and IQGAP1 might also have important implications for understanding the relationship between overexpression of cytoplasmic S100B and the development of a more aggressive cell phenotype during brain tumor progression. In astroglial brain tumors, progression from low grade (astrocytoma) into faster growing, more
dysplastic and invasive high grade tumors (glioblastomas), correlates with increased expression of cytoplasmic S100B (42). Although a causal relationship between S100B concentration and malignancy has not been demonstrated, it is hypothesized that increased concentration of S100B may contribute to neoplastic transformation. In these brain tumor cells, overexpressed S100B could interfere with the regulatory function of IQGAP1 during processes of dynamic rearrangement of cell-cell adhesion (23, 24) to favor cell motility and metastasis.

Acknowledgments—We thank Dr. J. Erickson for helpful discussion and the generous gift of IQGAP1 cDNAs and IQGAP1 antibodies, N. Assard for technical assistance, Mbélé T. and Mbélé A. for encouragements, and Drs. J. LaMarre and C. Benaud for the critical reading and comments on the manuscript.

REFERENCES
1. Schafer, B. W., and C. Heizmann. (1996). Trends Biochem. Sci. 21, 134–140
2. Baudier, J., Glasser, N., and Gerard, D. (1986) J. Biol. Chem. 261, 8192–8203
3. Haglid, K. G, Hamberger, A., Hansson, H. A., Hyden, H., Persson, L., Ronnback, L. (1975) Nature 258, 748–749
4. Rickmann, M., and Wolff, J. R. (1995) Histochemistry 103, 155–145
5. Scotto, C., Delphin, C., Deloulme, J. C., and Baudier, J. (1999) Mol. Cell. Biol. 19, 7168–7180
6. Selin-Lindahl, R. H., Barger, S. W., Welsh, M. J., and Van Eldik, L. J. (1990) J. Cell Biol. 111, 2021–2028
7. Xiong, Z., O'Hanlon, D., Becker, L. E., Roder, J., MacDonald, J. F., and Marks, A. (2000) Exp. Cell Res. 257, 281–289
8. Donato, R. (2001) Int. J. Biochem. Cell Biol. 33, 637–668
9. Nishiya, T., Knoepfl, T., Endo, S., and Itohara, S. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 4037–4042
10. Griffin, W. S., Stanley, L. C., Ling, C., White, L., MacLeod, V., Perret, L. J., White, C. L., and Araoz, C. (1989) Proc. Natl. Acad. Sci. U.S.A. 86, 7611–7615
11. Hofmann, M. A., Drury, S., Fu, C., Qu, W., Tabuchi, A., Lu, Y., Avila, C., Kamnham, N., Bierhaus, A., Kawajiri, A., Neurath, M. F., Slattery, T., Beach, D., McClintock, K. A., Van Eldik, L. J., and Baudier, J. (2001) J. Biol. Chem. 276, 30819–30826
12. Davey, G. E., Murrmann, P., and Heizmann, C. W. (2001) J. Biol. Chem. 276, 30819–30826
13. Pesic, E. R., Griffin, W. S., Akama, K. T., Raskind, M. A., and Van Eldik, L. J. (2001) Neurochem. Int. 39, 409–413
14. Pertela, L. V., Breun, J. C., Walz, R., Bianchin, M., Tort, A. B., Canabarro, U. P., Beheregayrat, S., Maruca, J. A., Xavier, B. M., Noto, E. C, Gonzalez, C. A., and Souza, D. O. (2002) Clin. Diagn. Lab. Immunol. 9, 164–166
15. Abdul-Khaliq, H., Schubert, S., Stoltenburg-Didinger, G., Troitsch, D., Böttcher, W., Hubler, M., Meissler, M., Gross-Siestrop, C., Alex–Mekishvili, V., Hetzer, R., and Lage, P. E. (2000) Clin. Chem. Lab. Med. 38, 1169–1172
16. Baudier, J., Mechly-Rosen, D., Newton, A., Lee, S. H., Koshland, D. E., Jr., and Cole, R. D. (1987) Biochemistry 26, 2886–2893
17. Deloulme, J. C, Assard, N, Ouengue Mble, G, Mangin, C, Kubwano, R, and Baudier, J. (2000) J. Biol. Chem. 275, 35302–35310
18. Takahashi, M., Chameczuk, A., Hong, Y., and Jackowski, G. (1999) Clin. Chem. 45, 1307–1311
19. Bashour, A. M., Fullerton, A. T., Hart, M. J., and Bloom, G. S. (1997) J. Cell Biol. 137, 1555–1566
20. Erickson, J. W., Cerione, R. A., and Hart, M. J. (1997) J. Biol. Chem. 272, 24443–24447
21. Fukata, M., Kuroda, S., Fuji, K., Nakamura, T., Shoji, I., Matsuzura, Y., Okawa, K., Inawatso, A., Kikuchi, A., and Kaibuchi, K. (1997) Cell Biol. 272, 29579–29583
22. Fukata, M., Kuroda, S., Nakagawa, M., Kawajiri, I., 20h, N., Shoji, I., Matsuzura, Y., Yonehara, S., Fujisawa, H., Kikuchi, A., and Kaibuchi, K. (1997) J. Biol. Chem. 272, 24044–24050
23. Fukata, S., Fukata, M., Nakagawa, M., Fuji, K., Nakamura, T., Ooka, T., Izawa, I., Nagase, T., Numura, N., Tani, H., Shoji, I., Matsuzura, Y., Yonehara, S., and Kaibuchi, K. (1998) Science 281, 832–835
24. Li, Z., Kim, S. H., Huggins, J. M., Brenner, M. B., and Sacks, D. B. (1998) J. Biol. Chem. 273, 37885–37892
25. Mateer, S. C., McDaniel, A. E., Nicolas, Y., Habermann, G. M., Lin, M. J., Cromer, D. A., King, M. E., and Bloom, G. S. (2002) J. Biol. Chem. 277, 12324–12333
26. Fukata, M., Watanabe, T., Nritake, J., Nakagawa, M., Yamaga, M., Kuroda, S., Matsuzura, Y., Iwamatsu, A., Perez, F., and Kaibuchi, K. (2002) Cell 109, 873–885
27. Gentil, B., Delphin, C., Ouengue Mble, G., Deloulme, J. C., Ferro, M., Garin, J., and Baudier, J. (2001) J. Biol. Chem. 276, 23253–23261
28. Ho, Y. D., Joyal, J. L., Li, Z., and Sacks, D. B. (1999) J. Biol. Chem. 274, 464–470
29. Joyal, J. L., Annan, R. S., Ho, Y. D., Huddleston, M. E., Carr, S. A., Hart, M. J., and Sacks, D. B. (1997) J. Biol. Chem. 272, 15419–15425
30. Baudier, J., Glasser, N., and Duportail, G. (1986) Biochemistry 25, 6934–6941
31. Rustandy, R. R., Baldissier, D. M., Drohat, A. C., and Weber, D. J. (1999) Protein Sci. 8, 1743–1751
32. McIntock, K. A., Van Eldik, L. J., and Shaw, G. S. (2002) Biochemistry 41, 5421–5428
33. Hart, M. J., Callow, M. G., Souza, B., and Polakis, P. (1996) EMBO J. 15, 2997–3005
34. Alexander, K., Wakim, B. T., Doyle, G. S., Walsh, K. A., and Storm, D. R. (1998) J. Biol. Chem. 263, 7544–7549
35. Baudier, J., Deloulme, J. C., Van Dorselaer, A., Black, D., and Matthes, H. W. (1991) J. Biol. Chem. 266, 229–237
36. Chakravarty, B., Morley, P., and Whitfield, J. (1999) Trends Neurosci. 22, 12–16
37. Delphin, C., Ronjak, M., Deloulme, J. C., Garin, G., Debussche, L., Higashimoto, Y., Nakagawa, M., and Baudier, J. (1999) J. Biol. Chem. 274, 10539–10544
38. Stemmel, J. M., Marchenko, N. D., Jimenez, G. S., Moll, U. M., Hope, T. J., and Wahl, G. M. (1999) EMBO J. 18, 1660–1672
39. Osman, M. A., and Cerione, R. A. (1998) J. Cell Biol. 142, 443–455
40. Zimmer, D. B., Cornwall, E. H., Reynolds, P. D., and Donald, C. M. (1998) J. Biol. Chem. 273, 4705–4711
41. Van Eldik, L. J., Jensen, R. A., Ehrenfried, B. A., and Whetsell, W. O. (1986) J. Histochem. Cytochem. 34, 977–982

Interaction of S100B Protein with IQGAP1

50007