A novel type of regulatory protein for the GDP/GTP exchange reaction of smg p25A, a ras p21-like small GTP-binding protein, in bovine brain cytosol.

Sasaki, T

Kobe J. Med. Sci. 37, 129/145, June 1991

A NOVEL TYPE OF REGULATORY PROTEIN FOR THE GDP/GTP EXCHANGE REACTION OF *smg* p25A, A *ras* p21-LIKE SMALL GTP-BINDING PROTEIN, IN BOVINE BRAIN CYTOSOL*

Takuya SASAKI

First Division, Department of Biochemistry,
Kobe University School of Medicine,

INDEXING WORDS

smg p25A; *ras* p21; the GDP/GTP exchange protein

SYNOPSIS

A regulatory protein for *smg* p25A, a *ras* p21-like small GTP-binding protein, was purified to near homogeneity from bovine brain cytosol. This regulatory protein, named *smg* p25A GDP dissociation inhibitor (GDI), regulated the GDP/GTP exchange reaction of *smg* p25A by inhibiting the dissociation of GDP from and the subsequent binding of GTP to it. Both the GDP- and GTP-bound forms of *smg* p25A bound to synaptic plasma membranes and vesicles. *smg* p25A GDI formed a complex with the GDP-bound form of *smg* p25A and thereby inhibited its binding to membranes and furthermore in-

---

*This article is dissertation submitted by Takuya Sasaki to Kobe University School of Medicine for the requirement of Doctor of Medical Sciences.

**Abbreviation used are: G proteins, GTP-binding proteins; GAP, GTPase-activating protein; *smg* p25A GDI, *smg* p25A GDP dissociation inhibitor; DTT, dithiothreitol; GTPγS, guanine 5'-(3-O-thio)triphosphate;EGTA,[ethlenebis(oxyethylenenitrilo)]tetraacetic acid; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Received for publication : March 22, 1991

Author's name in Japanese : 佐々木卓也

duced the dissociation of the prebound protein from the membranes. These results indicate that *smg* p25A GDI is a novel type of regulatory protein for the GDP/GTP exchange reaction and the intracellular translocation of *smg* p25A.

INTRODUCTION

smg p25A was first isolated from bovine brain membranes as a member of the superfamily of *ras* p21/*ras* p21-like small G proteins**.[8] *smg* p25A is composed of 220 amino acids with a calculated Mr value of 24,954.[11] *smg* p25A has the consensus amino acid sequences for GDP/GTP-binding and GTPase activities and the purified protein indeed exhibits these activities.[8,11] *smg* p25A was found to be identical with the protein encoded by the *rab3A* cDNA.[11,26,27] Among the *ras* p21-like small G proteins, *smg* p25A shows the highest amino acid sequence homology with the *Saccharomyces cerevisiae SEC4* and *YPT1* proteins, which are involved in the secretory processes of yeast.[7,19]

smg p25A is found in secretory cells with a regulated secretion type, such as neurons, exocrine cells, and endocrine cells, and is not found in secretory cells with a constitutive secretion type, such as liver and lymphocytes, or nonsecretory cells, such as skeletal muscles and fat cells.[3,4,13,14,15,17] Particularly, in neurons, *smg* p25A is abundant in presynapses where it is found in synaptic membranes, vesicles, and cytosol.[4,10,14,15] These results suggest that *smg* p25A plays important roles in regulated secretory processes.

The modes of activation and action of *smg* p25A have not been clarified. It is conceivable, however, by analogy with *ras* p21s (for a review, see Refs. 1 and 21) that *smg* p25A has GDP-bound inactive and GTP-bound active forms and that there are a protein converting the inactive form to the active form and an effector protein whose function is modulated by the active form. None of these proteins directly interacting with *smg* p25A, however, has been identified. Recently, a protein that stimulates the GTPase activity of c-*ras* p21s, designated as a GAP for c-*ras* p21s, has

been identified, purified, and characterized.[6,22] Subsequently, GAPs for the *rho* protein and *smg* p21 have been partially purified and characterized.[5,9,23,24] Each GAP is specific for each G protein. However, a GAP for *smg* p25A has not yet been identified. Instead, another type of regulatory protein for *smg* p25A that interacts with the GDP-bound form of *smg* p25A and thereby inhibits the dissociation of GDP from and the subsequent binding of GTP to *smg* p25A has been isolated. This paper describes the purification and properties of this novel type of regulatory protein for *smg* p25A, which is tentatively designated as *smg* p25A GDI.

MATERIALS AND METHODS

Materials and chemicals

smg p25A and *rho*B p21 were purified from bovine brain membranes.[8,25] c-Ha-*ras* p21 was purified from c-Ha-*ras* p21-expressing *Escherichia coli* as described.[9] *smg p21*B was purified from human platelet membranes.[16] Synaptic plasma membranes and vesicles were prepared from rat brains as described.[12] [^{3}H]GDP (0.99 TBq/mmol) and [^{35}S]GTPγS (49.7 TBq/mmol) were from Du Pont-New England Nuclear. BA-85 nitrocellulose filters (pore size 0.45 μm) were obtained from Schleicher & Schuell. Other materials and chemicals were from commercial sources.

Assay for the dissociation of [^{3}H]GDP from smg p25A

The [^{3}H]GDP-bound form of *smg* p25A was first made by incubating *smg* p25A (2 pmol) for 20 min at 30°C in a mixture (25 μl) containing 20 mM Tris/HCl at pH 7.5, 1 mM L-α-dimyristoylphosphatidylcholine, 1 mM DTT, 5 mM $MgCl_2$, 10 mM EDTA, and 1 μM [^{3}H]GDP (0.9-1.2 x10^4 cpm/pmol). After this first incubation, $MgCl_2$ (1 μl) was added to give a final concentration of 20 mM, and the mixture was immediately cooled on ice to prevent the dissociation of [^{3}H]GDP from *smg* p25A. Each radiolabeled sample was incubated with or without *smg* p25A GDI (50 pmol) in a mixture (75 μl)

containing 20 mM Tris/HCl at pH 7.5, 1 mM DTT, 1.35 μM GTP, and 10.1 mM EDTA or 10.1 mM $MgCl_2$, and the second incubation was performed for the indicated periods of time at 30°C. The free Mg^{2+} concentration in the second incubation mixture was 0.5 μM or 10 mM. About 2 ml of ice-cold 20 mM Tris/HCl at pH 7.5 containing 25 mM $MgCl_2$ and 100 mM NaCl was added to the reaction mixture followed by rapid filtration on nitrocellulose filters and washed with the same solution four times. The radioactivity was counted as described.[8)]

Assay for the binding of [^{35}S]GTPγS to smg p25A

The GDP-bound form of *smg* p25A (2 pmol) was first made as described above except that non-radioactive GDP was used instead of [^{3}H]GDP. After this first incubation, $MgCl_2$ (1 μl) was added to give a final concentration of 20 mM, and the mixture was immediately cooled on ice. Each sample was incubated with or without *smg* p25A GDI (50 pmol) as described above except that [^{35}S]GTPγS (0.9-1.2 x 10^4 cpm/pmol) was used instead of non-radioactive GTP. The second incubation was performed for the indicated periods of time at 30°C. The reaction was stopped as described above.

Complex formation of smg p25A GDI with the GDP-bound form of smg p25A

smg p25A (30 pmol) was incubated with [^{3}H]GDP and [^{35}S]GTPγS (2 x 10^4 cpm/pmol) for 20 min at 30°C to produce the [^{3}H]GDP-bound and [^{35}S]GTPγS-bound forms, respectively, as described above. Each radiolabeled sample (30 pmol) was mixed with or without *smg* p25A GDI (670 pmol) in a reaction mixture (300 μl) containing 20 mM Tris/HCl at pH 7.5, 1 mM GTP, 10 mM $MgCl_2$, 2.5 mM EDTA, 1 mM DTT, 250 μM L-α-dimyristoylphosphatidylcholine, and 0.06% CHAPS, and subjected to 4.8-ml continuous sucrose density ultracentrifugation at 100,000 x g for 1 hr. *smg* p25A GDI alone was separately subjected to the same ultracentrifugation. After the centrifugation, fractions of 150 μl each were collected from the bottom.

The amount of *smg* p25A was measured by counting the radioactivity of a 40-μl aliquot from each fraction after filtration on nitrocellulose filters as described above. The amount of *smg* p25A GDI was measured by SDS-PAGE followed by protein staining with silver.

Binding of the [^{3}H]GDP- and [^{35}S]GTPγS-bound forms of smg p25A to the synaptic plasma membranes and vesicles

The [^{3}H]GDP- and [^{35}S]GTPγS-bound forms of *smg* p25A were first formed as described above. Each radiolabeled sample (27 pmol) was incubated for 5 min at 30°C with synaptic plasma membranes (27 μg of protein) or vesicles (42 μg of protein) in the presence and absence of *smg* p25A GDI (940 pmol) in 500 μl of a reaction mixture (25 mM Tris/HCl at pH 7.5, 5 mM $MgCl_2$, 1 mM DTT, and 100 μM GDP for the [^{3}H]GDP-bound form or 100 μM GTP for the [^{35}S]GTPγS-bound form, respectively). After the incubation, 400 μl of each mixture was centrifuged on a discontinuous sucrose density gradient at 100,000 x g for 1 hr. After the centrifugation, fractions of 200 μl each were collected. The radioactivity of 100-μl aliquot of each fraction was counted as described above.

Determination.

Protein concentrations were determined with bovine serum albumin as a reference protein.[2)]

RESULTS

Purification of smg p25A GDI

All the purification procedures were performed at 0-4°C. *smg* p25A GDI was assayed by measuring the inhibition of the dissociation of [^{3}H]GDP from *smg* p25A during the following purification procedures. Cerebral tissue (450 g) was homogenized in a Waring blender with 450 ml of Buffer A (25 mM Tris/HCl at pH 7.5, 1 mM DTT, 1 mM $MgCl_2$, and 1 mM EGTA) containing 10 μM (*p*-

amidinophenyl)methanesulfonyl fluoride. The homogenate was further homogenized in a Potter-Elvehjem Teflon-glass homogenizer and centrifuged at 100,000 x g for 60 min. The supernatant was applied to a DEAE-Sephacel column (7.5 x 18 cm) equilibrated with Buffer A. After the column was washed with 8 l of Buffer A and 400 ml of Buffer A containing 0.3 M NaCl, *smg* p25A GDI was eluted by 800 ml of Buffer A containing 0.3 M NaCl. *smg* p25A GDI in the eluate was recovered in the 40-60% fraction by ammonium sulfate fractionation. This precipitate was dissolved in 26 ml of Buffer B (25 mM Tris/HCl at pH 7.5, 1 mM DTT, and 0.5 mM EDTA) and dialyzed overnight against Buffer B. One fourth of the supernatant was supplemented with 2.7 ml of Buffer B containing 4% sodium cholate. The sample was applied to a Mono Q column (1 x 10 cm) equilibrated with Buffer B containing 1% sodium cholate. After the column was washed with 190 ml of the same buffer, the elution was performed with a 180-ml linear gradient of NaCl (0-0.5 M) in

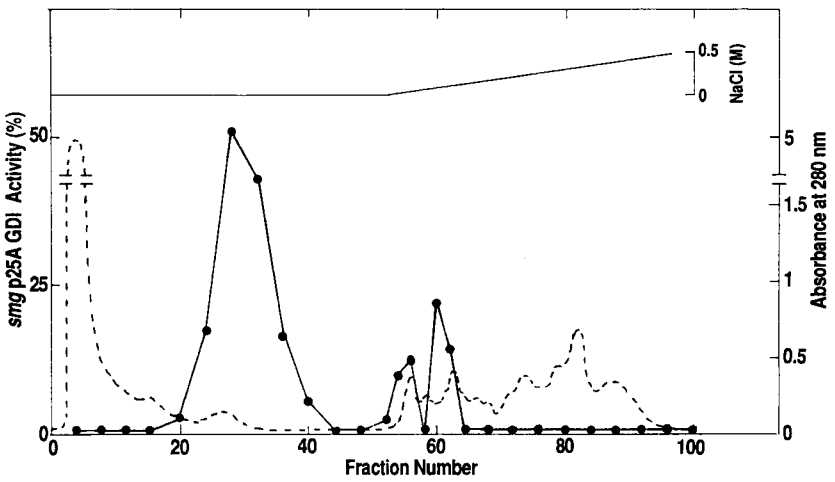

Fig. 1. Mono Q column chromatography of *smg* p25A GDI.
An aliquot (10 µl) of the indicated fractions was assayed for the *smg* p25A GDI activity to inhibit of the dissociation of [^{3}H]GDP from *smg* p25A. The activity of the assay sample was expressed as percent inhibition of the dissociation of [^{3}H]GDP from *smg* p25A. (●), the radioactivity of the [^{3}H]GDP-bound form of *smg* p25A; (---), absorbance at 280 nm; (——), NaCl concentration.

Buffer B containing 1% sodium cholate, and fractions of 4 ml each were collected. One major and two minor peaks of *smg* p25A GDI appeared in Fractions 25-34, 54-56 and 60-62 (Fig. 1). The relationship among these three peaks is not known, but the first major peak was studied here. The active fractions of the first peak were collected and concentrated to 5 ml by an Amicon ultrafiltration cell equipped with a PM-10 filter. The concentrate was dialyzed against Buffer B and used as a purified sample. The rest of the supernatant was subjected to the same Mono Q column chromatography in a similar manner.

Physical and kinetic properties of smg p25A GDI

The purified sample of *smg* p25A GDI was apparently pure on SDS-PAGE (Fig. 2). The Mr value of *smg* p25A GDI was estimated to be about 54,000 by SDS-PAGE. *smg* p25A GDI showed an S value of about 4.2 which corresponded to a Mr value of 59,000 (Fig. 3).

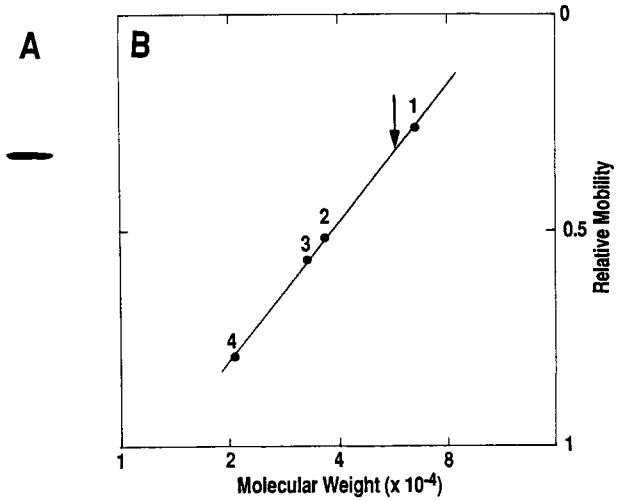

Fig. 2. Analysis of *smg* p25A GDI on SDS-PAGE. **A**, protein staining. About 20 pmol of *smg* p25A GDI was subjected to SDS-PAGE (12% polyacrylamide) followed by protein staining with Coomassie Brilliant Blue. **B**, The protein markers used were: 1, bovine serum alubmin (Mr=66,000); 2, glyceraldehyde-3-phosphate dehydrogenase (Mr=36,000); 3, carbonic anhydrase (Mr=29,000); 4, trypsine inhibiter (Mr=20,100). The arrow indicates the position of *smg* p25A GDI.

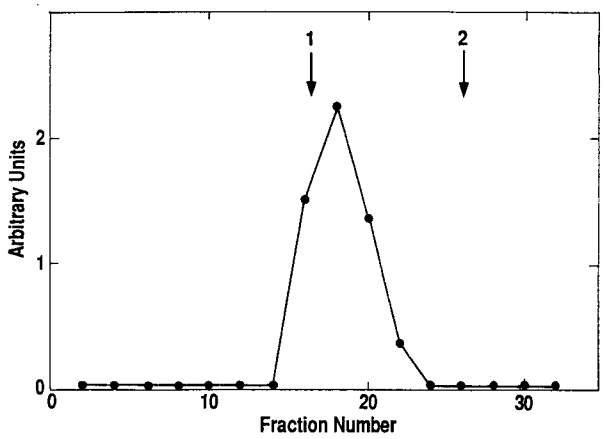

Fig. 3. Estimation of the Mr value of *smg* p25A GDI on continuous sucrose density gradient ultracentrifugation. *smg* p25A GDI (600 pmol) was subjected to the continuous sucrose density gradient. The ultracentrifugation was performed at 219,000 x g for 13.8 hr using 4.8 ml of a continuous sucrose density gradient. The amounts of *smg* p25A GDI were measured by SDS-PAGE followed by protein staining with silver. *Arrows 1* and *2* indicate the positions of human hemoglobin (Mr=64,000) and horse myoglobin (Mr=17,000), respectively.

[^{3}H]GDP was dissociated from *smg* p25A in a time-dependent manner (Fig. 4**A**). The initial velocity of this reaction was faster at 0.5 μM Mg^{2+} than at 10 mM Mg^{2+}. *smg* p25A GDI inhibited this dissociation at both Mg^{2+} concentrations. This action was dependent on the doses of GDI (Fig. 4**C**). [^{35}S]GTPγS was not dissociated from *smg* p25A irrespective of the presence of GDI at either Mg^{2+} concentration. [^{35}S]GTPγS bound to *smg* p25A more rapidly at 0.5 μM Mg^{2+} than at 10 mM Mg^{2+} (Fig. 4**B**). GDI inhibited this binding at both Mg^{2+} concentrations. This action was dependent on the doses of GDI (Fig. 4**C**). The doses of GDI necessary for inhibiting the dissociation of [^{3}H]GDP from and the binding of [^{35}S]GTPγS to *smg* p25A were the same (Fig. 4**C**).

smg p25A GDI did not stimulate the GTPase activity of *smg* p25A and by itself showed neither [^{35}S]GTPγS-binding nor GTPase activity (data not shown).

Complex formation of smg p25A GDI with the GDP-bound form of smg p25A

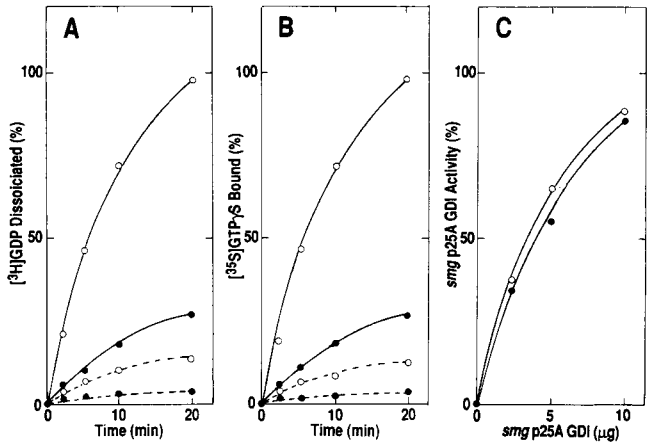

Fig. 4. Effect of *smg* p25A GDI on the dissociation of [^{3}H]GDP from and the binding of [^{35}S]GTPγS to *smg* p25A. **A**, effect of *smg* p25A GDI on the dissociation of [^{3}H]GDP from *smg* p25A. **B**, effect of *smg* p25A GDI on the binding of [^{35}S]GTPγS to *smg* p25A. (——), at 0.5 μM Mg^{2+}; (---), at 10 mM Mg^{2+}. (●), in the presence of GDI; (○), in the absence of GDI. **C**, dose-dependent effect of *smg* p25A GDI on the dissociation of [^{3}H]GDP from and the binding of [^{35}S]GTPγS to *smg* p25A. The *smg* p25A GDI activities were measured at 0.5 μM Mg^{2+} with various doses of GDI. The activities were expressed as percent inhibition of the dissociation of [^{3}H]GDP from and the binding of [^{35}S]GTPγS to *smg* p25A. (●), *smg* p25A GDI activity to inhibit the dissociation of [^{3}H]GDP from *smg* p25A; (○), *smg* p25A GDI activity to inhibit the binding of [^{35}S]GTPγS to *smg* p25A. The results shown are representative of three independent experiments.

The GDP-bound form of *smg* p25A appeared as a single peak with a Mr value of about 140,000 on sucrose density gradient ultracentrifugation (Fig. 5**A**). The Mr value of *smg* p25A estimated by SDS-PAGE and calculated by its primary structure were about 24,000 and 25,000, respectively.[8,11] Therefore, this peak appeared to be an aggregated form of *smg* p25A. *smg* p25A GDI appeared as a single peak at the position with a Mr value of 59,000 as described above (Figs. 3 and 5**A**). When *smg* p25A GDI was first mixed with the GDP-bound form of *smg* p25A and the mixture was then subjected to the same centrifugation, *smg* p25A at the position with a Mr value of 140,000 disappeared and that at the position with a Mr value of 85,000 appeared (Fig. 5**A**). Concomitantly, *smg* p25A GDI at the position with a Mr value of 59,000 partly shifted to the position

with a Mr value of 85,000. The partial shift was due to the addition of a large excess of *smg* p25A GDI against *smg* p25A. In contrast, when the mixture of *smg* p25A GDI with the GTPγS-bound form of *smg* p25A was subjected to the same centrifugation, *smg* p25A appeared at the original position (Fig. 5**B**). These results indicate that *smg* p25A GDI formed a complex with only the GDP-bound form of *smg* p25A.

Specificity of smg p25A GDI on other ras p21-like small G proteins

When *smg* p25A GDI was assayed for the activities to inhibit the dissociation of [^{3}H]GDP from and the binding of [^{35}S] GTPγS to c-Ha-*ras* p21, *rho*B p21, or *smg* p21B, *smg* p25A GDI was not active for any of these small G proteins (data not shown). *smg* p25A GDI did not form the complex with any of c-Ha-*ras* p21, *rho*B p21, or *smg* p21B (data not shown). These results indicate that *smg* p25A GDI is active for *smg* p25A and inactive for other small G proteins including c-Ha-*ras* p21, *rho*B p21, and *smg* p21B.

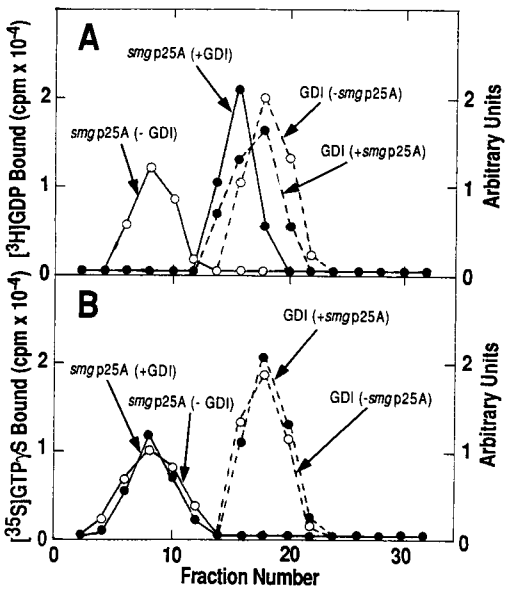

Fig. 5. Complex formation of *smg* p25A GDI with the GDP-bound form of *smg* p25A. **A**. the [^{3}H]GDP-bound form of *smg* p25A. **B**. the [^{35}S]GTPγS-bound form *smg* p25A. (——), *smg* p25A; (----), *smg* p25A GDI

Inhibition by smg p25A GDI of the binding of the [^{3}H]GDP-bound form of smg p25A to the synaptic plasma membranes and vesicles

When the [^{3}H]GDP- or [^{35}S]GTPγS-bound form of *smg* p25A was incubated with synaptic plasma membranes or vesicles and each mixture was subjected to the discontinuous sucrose density gradient ultracentrifugation to separate *smg* p25A in the membrane and soluble fractions, both forms of *smg* p25A were mostly recovered in the membrane fraction (Figs. 6, **A** and **B**). However, when each form of *smg* p25A was incubated with the membranes or vesicles in the presence of *smg* p25A GDI and the mixture was then subjected to the same centrifugation, the [^{3}H]GDP-bound form was recovered in the soluble fraction, but the [^{35}S]GTPγS-bound form was recovered in the membrane fraction (Figs. 6, **A** and **B**). Under these conditions, all amount of *smg* p25A GDI was recovered in the soluble fraction. These results indicate that *smg* p25A GDI inhibits the binding of the GDP-bound form of *smg* p25 to synaptic plasma membranes and vesicles.

Inhibition by smg p25A GDI of the dissociation by smg p25A GDI of exogenous and endogenous smg p25A from the synaptic plasma membranes and vesicles

When synaptic plasma membranes or vesicles which prebound the [^{3}H]GDP- or [^{35}S]GTPγS-bound form of exogenous *smg* p25A were incubated with *smg* p25A GDI and the mixture was subjected to the same centrifugation, only the [^{3}H]GDP-bound form was recovered in the soluble fraction (Figs. 6, **C** and **D**). The endogenous G proteins in the membranes or vesicles were first labeled with either [^{3}H]GDP or [^{35}S]GTPγS and then incubated with *smg* p25A GDI followed by the same centrifugation (Figs 6, **E** and **F**). Only the radioactivity of [^{3}H]GDP in the membrane fraction decresed and that in the soluble fraction inversely increased in the presence of *smg* p25A GDI. These results indicate that *smg* p25A GDI induces the dissociation of the GDP-bound form of both exogenous and endogenous *smg* p25A from the membranes and vesicles.

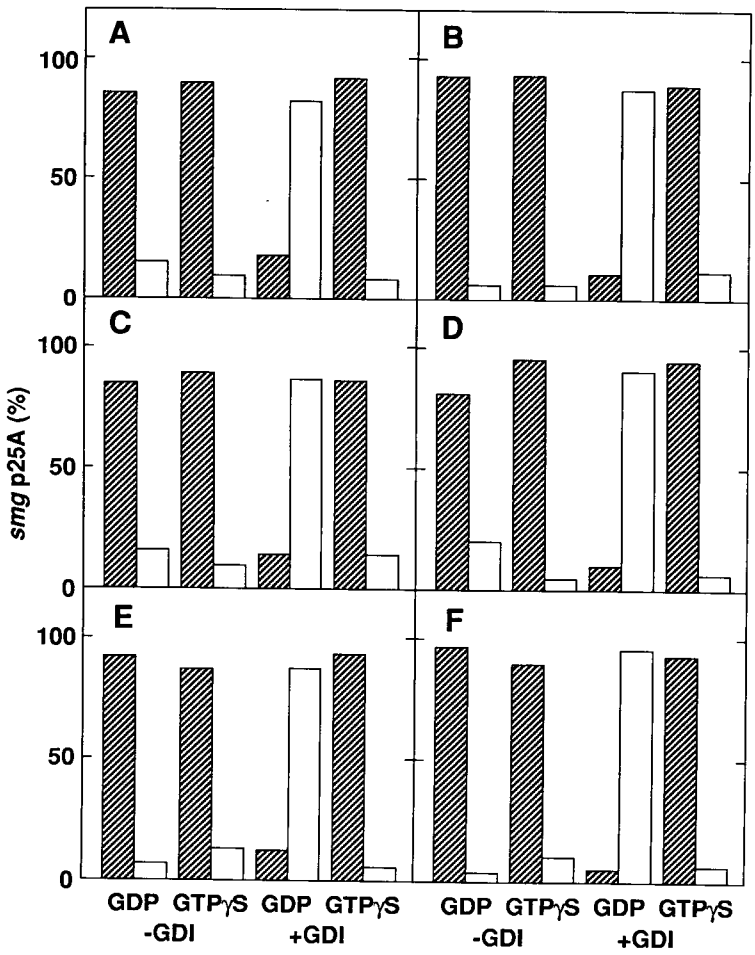

Fig. 6. Inhibition by *smg* p25A GDI of the binding of the [^{3}H]GDP-bound form of *smg* p25A to the synaptic plasma membranes and vesicles and dissociation by *smg* p25A GDI of exogenous and endogenous *smg* p25A from the synaptic plasma membranes and vesicles. **A** and **B**, inhibition by *smg* p25A GDI of the binding of the GDP-bound form of *smg* p25A to the synaptic plasma membranes and vesicles. **C** and **D**, dissociation by *smg* p25A GDI of the GDP-bound form of exogenous *smg* p25A from the synaptic plasma membranes and vesicles. **E** and **F**, dissociation by *smg* p25A GDI of the GDP-bound form of endogenous *smg* p25A from the synaptic plasma membranes and vesicles. **A**, **C** and **E**, synaptic plasma membranes. **B**, **D** and **F**, synaptic vesicles. (▨), membrane fraction; (▭), soluble fraction.

DISCUSSION

In the present studies, a novel type of regulatory protein for *smg* p25A has been purified from bovine brain cytosol. The Mr value of *smg* p25A GDI is estimated to be about 54,000 by SDS-PAGE and about 59,000 from the S value. *smg* p25A GDI inhibits the dissociation of GDP from and the subsequent binding of GTP to *smg* p25A. *smg* p25A GDI does not stimulate the GTPase activity of *smg* p25A and by itself shows neither GTP-binding nor GTPase activity. *smg* p25A GDI is active for *smg* p25A and inactive for other small G proteins such as c-Ha-*ras* p21, *rho*B p21, and *smg* p21B. *smg* p25A GDI forms a complex with only the GDP-bound form of *smg* p25A, but not with the GTP-bound form. Therefore, it is likely that *smg* p25A GDI first forms a complex with the GDP-bound form of *smg* p25A and thereby inhibits the disssociation of GDP from and the subsequent binding of GTP to *smg* p25A. In addition to this regulatory function of *smg* p25A, it is shown here that *smg* p25A GDI regulates the reversible binding of the GDP-bound form of *smg* p25A to the synaptic plasma membranes and vesicles. Both the GDP- and GTP-bound forms of *smg* p25A bind to the synaptic membranes and vesicles. *smg* p25A GDI inhibits this binding of only the GDP-bound form. Moreover, *smg* p25A GDI induces the dissociation of the GDP-bound form of endogenous and exogenous *smg* p25A from the synaptic membranes and vesicles. Therefore, it is likely that *smg* p25A GDI forms a complex with the GDP-bound form of *smg* p25A and thereby inhibits its binding to and stimulates its dissociation from the synaptic plasma membranes and vesicles.

On the basis of these observations, the mode of activation of *smg* p25A by *smg* p25A GDI is possible as follows. *smg* p25A is present in the cytoplasm in the GDP-bound inactive form, which is complexed with *smg* p25A GDI. When an unidentified signal comes to either *smg* p25A or *smg* p25A GDI, *smg* p25A is dissociated from *smg* p25A GDI, is converted to the GTP-bound active form, and then binds to membranes, or first binds to membranes and is then converted to the GTP-bound active form. The GTP-bound active form interacts with the putative effector protein, which may be present

in the membranes. The GTP which is associated with *smg* p25A is hydrolyzed to GDP by its intrinsic GTPase activity to produce the GDP-bound inactive form. This form of *smg* p25A becomes complexed with *smg* p25A GDI, and the complex is then dissociated from the membranes and translocated to the cytoplasm.

Since *smg* p25A shows the highest amino acid sequence homology with the *SEC4* and *YPT1* proteins and exists in secretory cells with a regulated secretion type,[7,19] *smg* p25A may play important roles in regulated secretory processes. The secretory process is assumed as the vectorial transport of vesicles between different membrane compartments such as endoplasmic reticula, Golgi complexes, secretory vesicles, and plasma membranes.[20] The vectorial transport of vesicles is regulated presumably at three steps, the budding of the vesicles from the donor membranes, the targeting of the vesicles to the appropriate acceptor membranes, and the fusion of the vesicles with the membranes. Accumulating evidence suggests that unknown small G proteins are involved in the regulation of the vectorial transport of vesicles. Therefore, it is possible that the *smg* p25A GDI-regulated reversible binding of *smg* p25A to the vesicles mediates their vectorial transport.

ACKNOWLEDGEMENTS

The author would like to express his sincere gratitude to Prof. Y. Takai (First Division, Department of Biochemistry, Kobe University School of Medicine) and Prof. H. Nakamura (Depertment of Pedeatrics, Kobe University School of Medicine) for valuable discussion, encouragement and support in this work.

REFERENCES

1. Barbacid, M.: Annu. Rev. Biochem. 1987. 56. 779/827. *ras* genes.
2. Bradford, M. M.; Anal. Biochem. 1976. 74. 284/254. A rapid and sensitive method for the determination of microgram quantities of protein utilizing the principle of protein-dye binding.

3. Burstein, E. and Macara, I.G.: Mol. Cell. Biol. 1989. 9. 4807/4811. The *ras*-like protein $p25^{rab3A}$ is partially cytosolic and is expressed only in neural tissue.
4. Darchen, F., Zahraoui, A., Hammel, F., Monteils, H.P., Tavitian, A. and Scherman, D.: Proc. Natl. Acad. Sci. U.S.A. 1990. 87. 5692/5696. Association of the GTP-binding protein Rab3A with bovine adrenal chromaffin granules.
5. Garrett, M.D., Self, A.J., Oers, C.V. and Hall, A.: J. Biol. Chem. 1989. 264. 10/13. Identification of distinct cytoplasmic targets for *ras*/R-*ras* and *rho* regulatory proteins.
6. Gibbs, J.B., Schaber, M.D., Allard, W.J., Sigal, I.S. and Scolnick, E.M.: Proc. Natl. Acad. Sci. U.S.A. 1988. 85.5026/5030. Purification of *ras* GTPase activating protein from bovine brain.
7. Goud, B., Salminen, A., Walworth, N.C. and Novick, P.J.: Cell. 1988. Cell. 53. 753/768. A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast.
8. Kikuchi, A., Yamashita, T., Kawata, M., Yamamoto, K., Ikeda, K., Tanimoto, T. and Takai,Y.: J. Biol. Chem. 1988. 263. 2897/2904. Purification and characterization of a novel GTP-binding protein with a molecular weight of 24,000 from bovine brain membranes.
9. Kikuchi, A., Suzuki, T., Araki, S., Hata, Y. and Takai, Y.: J. Biol. Chem. 1989. 264. 9139/9136. Purification and characterization from bovine brain cytosol of two GTPase-activating proteins specific for *smg* p21, a GTP-binding protein having the same effector domain as c-*ras* p21s.
10. Kim, S., Kikuchi, A., Mizoguchi, A. and Takai, Y.: Mol. Brain Res. 1989. 6. 167/176. Intrasynaptosomal distribution of the *ras*, *rho* and *smg*-25A GTP-binding proteins in bovine brain.
11. Matsui, Y., Kikuchi, A., Kondo, J., Hishida, T., Teranishi, Y. and Takai, Y.: J. Biol. Chem. 1989. 263. 11071/11074. Nucleotide and deduced amino acid sequences of a GTP-binding protein family with molecular weight of 25,000 from bovine brain.

12. Mizoguchi, A., Ueda, T., Ikeda, K., Shiku, H., Mizoguti, H. and Takai, Y.: Mol. Brain Res. 1989. 5. 31/44. Localization and subcellular distribution of cellular *ras* gene products in rat brain.
13. Mizoguchi, A., Kim, S., Ueda, T. and Takai, Y.: Biochem. Biophys. Res. Commun. 1989. 162. 1438/1445. Tissue distribution of *smg* p25A, a *ras* p21-like GTP-binding protein, studied by use of a specific monoclonal antibody.
14. Mizoguchi, A., Kim, S., Ueda, T., Kikuchi, A., Yorifuji, H., Hirokawa, N. and Takai, Y.: J. Biol. Chem. 1990. 265. 11872/11879. Localization and subcellular distribution of *smg* p25A, a ras p21-like GTP binding protein, in rat brain.
15. Mollard, G.F., Mignery, G.A., Baumert, M., Perin, M.S., Hanson, T.J., Burger, R., Jahn, R. and Südhof, T.C.: Proc. Natl. Acad. Sci. 1990. 87. 1988/1992. rab3 is a small GTP-binding protein exclusively localized to synaptic vesicles.
16. Ohmori, T., Kikuchi, A., Yamamoto, K., Kim, S. and Takai, Y: J. Biol. Chem. 1989. 264. 1877/1881. Small molecular weight GTP-binding proteins in human platelet membranes.
17. Olofsson, B., Chardin, P., Touchot, N., Zahraoui, A. and Tavitian, A.: Oncogene. 1988. 3. 231/234. Expression of the *ras*-related *ral*A, *rho*12 and *rab* genes in adult mouse tissues.
18. Sano, K., Kikuchi, A., Matsui, Y., Teranishi, Y. and Takai, Y.: Biochem. Biophys. Res. Commun. 1989. 158. 377/385. Tissue-specific expression of a novel GTP-binding protein (*smg* p25A) mRNA and its increase by nerve growth factor and cyclic AMP in rat pheochromocytoma PC-12 cells.
19. Segev, N., Mulholland, J. and Botstein, D.: Cell. 1988. 52. 915/924. The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretory machinery.
20. Pfeffer, S.R. and Rothman, J.E.: Annu. Rev. Biochem. 1987. 56. 829/852. Biosynthtic protein transport and sorting by the endoplasmic reticulum and golgi.
21. Takai, Y., Kaibuchi, K., Kikuchi, T. and Kawata, M.: FASEB J. in press. A superfamily of small GTP-binding proteins Functions and modes of action.

22. Trahey, M. and McCormick, F.: Science. 1987. 238. 542/545. A cytoplasmic protein stimulates normal N-*ras* p21 GTPase, but does not affect oncogenic mutants.
23. Ueda, T., Kikuchi, A., Ohga, N., Yamamoto, J. and Takai,Y.: Biochem. Biophys. Res. Commun. 1989. 159. 1411/1419. GTPase activating proteins for the *smg* p21 GTP-binding protein having the same effector domain as the *ras* proteins in human platelets.
24. Yamamoto, J., Kikuchi, A., Ueda, T., Ohga, N. and Takai, Y.: Mol. Brain Res. 1990. 8. 105/111. A GTPase-activating protein (GAP) for *rho*B p20, a *ras* p21-like GTP-binding protein——Partial purification , characterization and subcellular distribution in rat brain.
25. Yamamoto, K., Kondo, J., Hishida, T., Teranishi, Y. and Takai, Y.: J. Biol. Chem. 1988. 263. 9926/9932. Purification and characterization of GTP-binding protein with a molecular weight of 20,000 in bovine brain membranes - Identification as the *rho* gene product.
26. Zahraoui, A., Touchot, N., Chardin, P. and Tavitian, A.: Nucl. Acids Res. 1988. 16. 1204. Complete coding sequence of the *ras* related *rab*3 and *rab*4 cDNAs.
27. Zahraoui, A., Touchot, N., Chardin, P. and Tavitrian, A.: J. Biol. Chem. 1989. 264. 12394/12401. The human rab genes encoded a family of GTP-binding proteins related to yeast YPT1 and SEC4 protein involved in secretion.