In the present studies, we have purified a novel small M, GTP-binding protein, designated as smg p21, to near homogeneity from bovine brain crude membranes, isolated the complementary DNA (cDNA) of this protein from a bovine brain cDNA library, determined the complete nucleotide and deduced amino acid sequences, and characterized the kinetic properties. The cDNA of smg p21 has an open reading frame encoding a protein of 184 amino acids with a calculated M, of 20,987. The M, of purified smg p21 is estimated to be about 22,000 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Homology search indicates that smg p21 is a novel protein with the consensus amino acid sequences for GTP/GDP-binding and GTPase domains but shares about 55% amino acid sequence homology with the human c-Ha-ras protein. Moreover, smg p21 has the same putative effector domain as the Ha-, Ki-, and N-ras proteins at the same position and the same consensus C-terminal sequence as in these ras proteins. Consistent with these structural properties, smg p21 binds specifically [35S]guanosine 5'-triphosphate (GTPγS), GTP, and GDP with a K, value for GTPγS of about 40 nM. smg p21 binds about 0.7 mol of GTPγS/mol of protein. [35S]GTPγS-binding to smg p21 is inhibited by pretreatment with N-ethylmaleimide. smg p21 hydrolyzes GTP to liberate P, with a turnover number of about 0.007 min⁻¹. These kinetic properties of smg p21 are similar to those of the c-ras proteins. These results suggest that smg p21 is a novel GTP-binding protein exerting action(s) similar or antagonistic to that (those) of the ras proteins.

*The investigation in the Department of Biochemistry, Kobe University School of Medicine was supported by Grants-in-Aid for Scientific Research and Cancer Research from the Ministry of Education, Science, and Culture, Japan (1987, 1988), Grants-in-Aid for Abnormalities in Hormone Receptor Mechanisms (1987, 1988), Cardiovascular Diseases (1987, 1988), and for Cancer Research (1988) from the Ministry of Health and Welfare, Japan, and by grants from the Yamanouchi Foundation for Research on Metabolic Disease (1987, 1988), and for Cancer Research (1988). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J04196.

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The ras genes (Ha-, Ki-, and N-ras) encode an evolutionarily conserved family of proteins with M, values of about 21,000, generally designated as ras p21s, that are widely present in eukaryotic cells (for reviews, see Refs. 1 and 2). ras p21s exhibit both GTP/GDP-binding and GTPase activities (1, 2). The ras genes are activated by a single point mutation that most frequently affects the amino acid residue at either position 12, 59, or 61, and the activated genes can transform established cell lines (1, 2). These mutations cause the reduction of their GTPase activity (1, 2). Moreover, a single amino acid substitution at position 119 or 144 decreases the affinity of ras p21s for GTP (2). These observations together with x-ray crystallographic analysis of ras p21s have suggested that amino acid residues 10–16 and 57–62 are the domains binding the phosphate portion of guanine nucleotide and responsible for GTPase activity and that residues 113–119 and 143–147 are the domains binding the guanine portion (1, 2, for a review, see Refs. 3, and 4). These four domains are highly conserved and identical among three ras p21s (1, 2). In addition to these domains, ras p21s have unique consensus C-terminal sequences that is Cys-X-X-X, where X is any amino acid (1, 2). This cysteine residue is palmitoylated and ras p21s attach to plasma membranes through this fatty acid (2).

The mode of action of ras p21s has not definitely been clarified, but by analogy with G proteins serving as transducers for membrane receptors, such as G, G, G, and transducin (for reviews, see Refs. 5–8), it has been suggested that ras p21s possess two convertible forms, the GDP-bound inactive and GTP-bound active forms, and that there are converting proteins which convert the GDP-bound inactive form to the GTP-bound active form and effector proteins whose functions are modulated by the GTP-bound active form (1, 2). Consistent with this model, it has been shown that a single amino acid substitution at position 35, 36, 38, or 40 of activated ras p21s eliminates their transforming activity without affecting their GTP/GDP-binding activity, and it has been proposed that the region between residues 35 and 40 is the effector domain interacting with the effector proteins (2, 9). In fact, a protein activating the GTPase activity of c-ras p21s, designated as GAP, has recently been identified (10) and has been suggested to interact with the effector domain (11, 12).

In addition to the ras genes, several ras-like genes have
been identified in mammalian tissues and yeast. These include the rho, rol, R-ras, ypt1, rho2, and SEC4 genes encoding the proteins which share about 32, 56, 55, 32, 30 and 30% amino acid sequence homology with c-Ha-ras p21, respectively (13–18). These genes encode proteins with M, values about 20,000 and their deduced amino acid sequences include the sequences similar to those of the GTP/GDP-binding and GTPase domains of ras p21s but do not have the same sequence as that of the effector domain of ras p21s except for the R-ras protein (2, 13–18). The R-ras protein has the same amino acid sequence as that of the effector domain of ras p21s (15). Although the genes have not been identified, two G proteins with M, values of about 21,000 and 25,000, designated as ARF and G, respectively, have been purified from mammalian tissues (19–23). In this paper, these G proteins with M, values of about 20,000 are designated as small M, G proteins and G proteins with an agp subunit structure serving as transducers for membrane receptors are designated as large M, G proteins, since the M, values of their agp subunits with GTP-binding and GTPase activities are between 39,000 and 52,000 (5–8).

We have recently separated multiple small M, G proteins from bovine brain crude membranes (24–27). We have purified and characterized a novel small M, G protein with a M, of 24,000 (24). Then, we have cloned the DNA of M, 24,000 G protein and determined its nucleotide and deduced amino acid sequences (25). Moreover, we have isolated two other cDNAs encoding small M, G proteins highly homologous with M, 24,000 G protein (25). We have designated these M, 24,000 G protein as smg p25A and the two other G proteins as smg p25B and smg p25C (smg p25 stands for small molecular weight guanine nucleotide-binding protein with a M, of 25,000), since the M, values of these proteins calculated from their deduced amino acid sequences are near 25,000 (25). Smg p25A shares about 28% amino acid sequence homology with c-Ha-ras p21 (25). Subsequently, we have purified and characterized two other small M, G proteins with M, values of about 20,000 and 21,000, and identified M, 20,000 G protein and M, 21,000 G protein as the rho protein (rho p20) and c-Ki-ras p21, respectively (26, 27).

In the present studies, we have purified another small M, G protein to near homogeneity from bovine brain crude membranes. The DNA of this protein from a bovine brain cDNA library, determined the complete nucleotide and deduced amino acid sequences, and characterized the kinetic properties. Since the M, value of this small M, G protein calculated from its deduced amino acid sequence is about 21,000, we have designated it as the smg-21 protein (smg p21). Homolog search indicates that smg p21 is a novel small M, G protein with the consensus amino acid sequences for GTP/ GDP-binding and GTPase domains but shares 55% amino acid sequence homology with human c-Ha-ras p21. Moreover, smg p21 has the same putative effector domain as Ha-, Ki-, and N-ras p21s at the same position and the same consensus C-terminal sequence as in the ras p21s (1–4, 9). This paper describes the purification procedures and structural and kinetic properties of this novel small M, G protein.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—The crude membrane fraction was prepared from bovine brain as described previously (24). Achromobacter protease I was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). YMC pack AP-802 C1 column (4.6 × 150 mm) was purchased from Yamamura Chemical Lab. Co. (Kyoto, Japan). Other materials and chemicals were obtained from the same sources as described (24–26).

[^25][125]I GTPyS-binding and GTPase Assays—[^25][125]I GTPyS-binding to G proteins was determined by use of the nitrocellulose filter method as described (24). GTPase activity of smg p21 was estimated by the liberation of ^32P from [γ-^32P]GTP as described (24). Amino Acid Sequencing of smg p21—Purified smg p21 (25 μg of protein) was applied to a YMC pack AP-802 C1 column prequillibrated with 0.1% trifluoroacetic acid to remove sodium cholate which was included in the buffer of the purified preparation. The elution was performed with a 40-mL linear gradient of acetonitrile-2-propanol (0–100%, acetonitrile:2-propanol = 37:63) at a flow rate of 1 ml/min. Smg p21 was eluted at a retention time of about 25 min. Smg p21 prepared in this way was then completely digested with Achromobacter protease I and subjected to Bakerbond WP-octyl column chromatography under the conditions described previously (20). The peptides separated by this column chromatography were sequenced using an automated gas-phase sequencer (Applied Biosystems, Model 470A).

Molecular Cloning and Determination of the Nucleotide Sequence of the smg-21 cDNA—An oligonucleotide probe was synthesized using a DNA synthesizer (Applied Biosystems, Model 380A) according to the partial amino acid sequence determined from purified smg p21. The bovine brain cDNA library prepared previously (25) was screened by the method of Hanahan and Meselson (28). The obtained cDNA clone in the λgt10 phage vector was recloned using a pUC19 vector (29), and several deleted clones were constructed by the method of Sanger et al. (31). The nucleotide sequence was determined by the method of Sanger et al. (31). The computer program IDEAS was used for homology search (32) and the calculation for hydrophobicity (33).

Determination of the Radioactivities of ^32S- and ^32P-labeled samples were determined using a liquid scintillation system (Beckman, Model LS-801). Protein was determined by the method of Lowry et al. (34). Where specified, protein was alternatively determined with bovine serum albumin as a standard protein by densitometric tracing of protein bands stained with Coomassie Brilliant Blue on an SDS-polyacrylamide gel by the method of Weber et al. (35).

RESULTS

Purification—G proteins were extracted with sodium cholate from bovine brain crude membranes and purified by successive chromatographies of Ultrogel AcA-44, phenyl-Sepharose CL-4B, hydroxylapatite, and Mono Q HR5/5 columns under the same conditions as described previously (24, 26). Briefly, the extracted G proteins were subjected to gel filtration on an Ultrogel AcA-44 column. Two peaks of [ ^35S]GTPyS-binding activity appeared on this column chromatography. The first peak contained large M, G proteins including Ga, Gb, and Gc, and the second peak contained small M, G proteins. Small M, G proteins in this second peak were subjected to phenyl-Sepharose CL-4B column chromatography. A single peak of [ ^35S]GTPyS-binding activity appeared. The active fractions of this peak were subjected to hydroxylapatite column chromatography. Two peaks of [ ^35S]GTPyS-binding activity appeared. The second peak contained c-Ki-ras p21 (27). The active fractions of the first peak were collected, concentrated, and then half of the concentrate was subjected to Mono Q HR5/5 column chromatography. Four peaks of [ ^35S]GTPyS-binding activity appeared. The third peak contained rho p20 (26) while the fourth peak contained smg p25A (24, 25). The active fractions of the first peak (Fractions 4–11) were pooled and purified further. Another half of the concentrate was applied to a Mono Q HR5/5 column followed by the same procedures. The same active fractions of the first peak were pooled and purified further.

Two batches of the pooled fractions of the first peak of the Mono Q HR5/5 column chromatography (8 ml, 0.95 mg of protein) were combined and adjusted to pH 5.0 with 1 M sodium acetate at pH 4.0. Half of the pooled sample was then applied to a Mono S HR5/5 (0.5 × 5 cm) column prequillibrated with 50 mM sodium acetate at pH 5.0 containing 1 mM EDTA, 1 mM DTT, 5 mM MgCl2, and 0.6% CHAPS, followed by the application of another half of the sample to the same column. After the column was washed with 5 ml of the same buffer, the elution was performed with a 20-ml linear gradient.
of NaCl (0-1.0 M) in the same buffer at a flow rate of 0.5 ml/min. Fractions of 0.5 ml each were collected in tubes containing 33 μl of 1.5 M Tris/HCl at pH 9.0. When each fraction was assayed for [35S]GTPγS-binding activity, four peaks appeared as shown in Fig. 1. The active fractions of the fourth peak (Fractions 50-68) were pooled and purified further. The other peaks were not purified further here. ARF was detected in the third peak (Fractions 38-42) by immunoblot analysis (data not shown).

The pooled fractions of the fourth peak of the Mono S HR5/5 column chromatography (9.5 ml, 0.23 mg of protein) were diluted with 9 volumes of 20 mM Tris/HCl at pH 9.0 containing 1 mM EDTA, 1 mM DTT, 5 mM MgCl₂, and 0.5% sodium cholate to decrease the NaCl concentration, and then applied to a Mono Q HR5/5 (0.5 × 5 cm) column preequilibrated with the same buffer. After the column was washed with 5 ml of the same buffer, the elution was performed with a 20-ml linear gradient of NaCl (0-0.4 M) in the same buffer at a flow rate of 0.5 ml/min. Fractions of 0.5 ml each were collected. When each fraction was assayed for [35S]GTPγS-binding activity, three broad peaks (Fractions 10-20, 26-30, and 40-44) and one sharp peak (Fractions 32-38) appeared as shown in Fig. 2A. Fig. 2B shows silver staining of proteins of each fraction on SDS-polyacrylamide gels. The first broad peak was apparently homogeneous. The proteins corresponding to the β subunit (a Mr of 35,000 or 36,000) and the γ subunit (a Mr of about 10,000) of large Mr G proteins were not observed in this peak. The active fractions of the first peak (Fractions 10-20) were pooled and characterized. Other peaks were not purified further in this paper. The pooled sample of the first peak showed a single protein band on SDS-PAGE as shown in Fig. 3. The Mr of this protein was estimated to be about 22,000 by SDS-PAGE. However, the Mr of this protein was calculated to be about 20,987 from its deduced amino acid sequence as described below. Therefore, we designated this protein as smg p21. When smg p21 was kept frozen at -80°C, the GTP-binding activity was not lost at least for 1 week.

Amount in Bovine Brain Crude Membranes—A summary of typical purification of smg p21 is shown in Table I. About 11 μg of smg p21 was purified from 858 mg of protein in the cholate extract from the crude membrane fraction. It is difficult to calculate the exact yield of smg p21 through our purification procedures, since at least ten small Mr G proteins were separated by column chromatographies. However, on the assumption that each small Mr G protein is purified with the same yields during each column chromatography, the yield of the purification of smg p21 is calculated to be about 6.1%. Therefore, it could be estimated that about 0.02% of the total proteins in the cholate extract from the crude membrane fraction is smg p21. The amount of smg p21 in bovine brain crude membranes is comparable to those of rho p20 and c-Ki-ras p21 (26, 27), about one-fifth those of smg p25A (24, 25), ARF (19, 20), and G₁ (36), and over 50-fold less than those of G₁ and Gα (37, 38).

Amino Acid Sequencing—Purified smg p21 was completely identified as a new ras p21-like GTP-binding protein.
digested with *Achromobacter* protease I and subjected to Bakingbond WP-octyl column chromatography. Peptide peaks were monitored spectrophotometrically at 215 nm.

The nucleotide sequence of the cDNA and the amino acid sequence of human c-Ha-ras p21. The cDNA clone with a 2.5-kb long insert was obtained. The physical map of the insert and the sequencing strategy are shown in Fig. 5. Using this probe, approximately 3

**TABLE I**

| Purification step         | Total volume (ml) | Total protein (mg) | Total GTPyS-binding amount (nmol) | Specific activity (nmol/mg) | Yield (%) |
|---------------------------|-------------------|--------------------|-----------------------------------|-----------------------------|-----------|
| Cholate extract           | 95                | 858                | 484                               | 0.06                        | 100       |
| Uitrogel AcA-44 (second peak) | 200              | 90                 | 223                               | 2.5                         | 46        |
| Phenyl-Sepharose CL-4B    | 120               | 32                 | 142                               | 4.4                         | 29        |
| Hydroxylapatite (first peak) | 240              | 6.8                | 51                                | 7.5                         | 11        |
| Mono Q HR5/5 (first peak) | 8                 | 0.95               | 10                                | 8.6                         | 2.1       |
| Mono S HR5/5 (fourth peak) | 9.5              | 0.23               | 4.6                               | 20                          | 0.95      |
| Second Mono Q HR5/5 (first peak) | 5.5          | 0.011              | 0.36                              | 33                          | 0.074     |

* Determined by densitometric tracing of the gel after SDS-PAGE as described under "Experimental Procedures."

**FIG. 4. Peptide map of smg p21.** smg p21 (5 μg of protein) was digested with *Achromobacter* protease I. The digested protein was subjected to Bakingbond WP-octyl column chromatography. Peptide peaks were monitored spectrophotometrically at 215 nm.

The amino acid sequences of the peak 1–6 peptides were as follows: peak 1, Met-Arg-Glu-Tyr-Lys; peak 2, Tyr-Asp-Pro- Thr-Ile-Val-Gln-Phe-Val- Gly-Ile-Phe; peak 3, Glu- Glu-Glu-Asp-Phe- Tyr- Leu-Val-Gln-Phe- Val-Gly-Ile-Phe; peak 4, Ser-Ala-Leu-Thr-Val-Gln-Phe-Val-Gly- Ile-Phe; peak 5, Ile-Asn-Val-Ala-Glu-Ile-Phe-Tyr- Leu-Val-Glu-Val-Glu-Ile-Arg-Lys; peak 6, Asn-Gly-Gln-Gly-Phe-Ala-Leu-Val-Tyr-Ser-Ile-Thr-Ala-Gln-Ser-Thr- Phe-Asn-Glu-Leu-Asp-Leu-Glu-Asp-Leu-Glu-Val-Lys.

**Molecular Cloning and Determination of Nucleotide and Deduced Amino Acid Sequences**—An oligonucleotide probe, 5′-GC(A/G)AANC(5′)TTONCC(A/G)TT, was synthesized according to the amino acid sequence Asn-Gly-Glu-Gl y-Phe-Ala, which was a part of the peak 6 peptide shown in Fig. 4. Using this probe, approximately 5 × 10⁸ recombinant phage plaques from a bovine brain cDNA library were screened. One clone with a 2.5-kb long insert was obtained. The physical map of the insert and the sequencing strategy are shown in Fig. 5. Fig. 6 shows the nucleotide sequence of the cDNA and its deduced amino acid sequence in comparison with the amino acid sequence of human c-Ha-ras p21. The cDNA contained an open reading frame of 184 amino acids with a calculated M, of 20,987. The amino acid sequence correspond-

**FIG. 5. A restriction map and sequencing strategy of the smg-21 cDNA.** The coding region is indicated by an open box. The hatched box at the right end indicates poly(A) region. The arrow above in the box indicates the direction of translation. The arrows under the map indicate the direction and the region of DNA sequencing. bp, base pairs.

ing to the oligonucleotide probe used for the screening and the sequences of the five other peptides determined from purified smg p21, including the N-terminal peptide, were found in the amino acid sequence deduced from the cDNA, indicating that this amino acid sequence is the complete sequence of smg p21.

Homology search indicated that smg p21 was a novel small G protein but shared about 55, 23, 50, 47, 31, 33, 34, and 32% amino acid sequence homology with the human c-Haras, *Aplysia rho*, simian ral, human R-ras, mouse ypt1, rat rab2, yeast *SEC4*, and bovine smg-25-A proteins, respectively (2, 13–18, 25). smg p21 had the consensus amino acid sequences for GTP/GDP-binding and GTPase domains which are homologous with those found in other small M, G proteins (1–4, 13–18, 25). Particularly, smg p21 had the same amino acid sequence as that of the effector domain of Ha-, Ki-, and N-ras p21s, that is Cys-Val-Leu-(1, 2).

**Kinetic Properties**—Purified smg p21 bound [35S]GTPγS in time- and dose-dependent manners as shown in Figs. 7 and 8, respectively. Scatchard plot analysis showed that smg p21 bound maximally 0.7 ± 0.1 mol (mean ± S.E.) of this nucleotide/mol of protein with a Kd value of 40 ± 8 nM (mean ± S.E.). [35S]GTPγS binding to smg p21 was progressively inhibited by increasing concentrations of nonradioactive GTPγS, GTP, and GDP. Inhibitory potencies of these three nucleotides were similar. Other nucleotides such as ATP, CTP, and UTP were not effective. [35S]GTPγS-binding to smg p21 was inhibited by pretreatment with NEM. This inhibition was blocked by the simultaneous presence of DTT. smg p21 showed GTPase activity as estimated by the liberation of 32P from [γ-³²P]GTP. Fig. 9 shows the time course of
DISCUSSION

In our preceding papers, we have purified smg p25A, rho p20, and c-Ki-ras p21 to near homogeneity from bovine brain crude membranes and characterized them (24, 26, 27). Moreover, we have isolated the cDNA of smg p25A and two other cDNAs of smg p25B and smg p25C highly homologous with that of smg p25A and confirmed that smg p25s constitute a novel small M, G protein family (25). In the present studies, we have purified another small M, G protein to near homogeneity from bovine brain crude membranes, isolated the cDNA of this protein from a bovine brain cDNA library, determined the complete nucleotide and deduced amino acid sequences, and characterized the kinetic properties. Homology search indicates that this is a novel small M, G protein. Therefore, we have designated this small M, G protein as smg p21. smg p21 has characteristic structural and kinetic properties similar to those of ras p21s (Ha-, Ki-, and N-ras p21s). The most interesting structural property of smg p21 is that it possesses the same amino acid sequence as that of the effector domain of ras p21s (2, 9). Among various small M, G proteins, only the R-ras protein has the same amino acid sequence as that of the effector domain of ras p21s, but other small M, G proteins including the rho, rac, rob2, ypt1, SEC4,
smg-25A, smg-25B, and smg-25C proteins do not have the same amino acid sequence (13-18, 25). Moreover, among various small M, G proteins, smg p21 shows the highest amino acid sequence homology with ras p21s. Particularly, the first 60 amino acid residues of smg p21 share about 70% amino acid sequence homology with the same region of human c-Ha-ras p21. This region of c-Ha-ras p21 is identical to that of c-Ki- and N-ras p21s (1, 2). The residual region of smg p21 is far less homologous with that of c-Ha-ras p21 as well as with that of other ras p21s. The first 60 amino acid residues of c-Ha-ras p21 have been shown by x-ray crystallographic analysis to form three β strands, one α helix, and three loops (4). In this structure, the effector domain is exposed at the surface of the molecule. The fact that smg p21 has the same amino acid sequence as that of the effector domain of ras p21s at the same position in the highly homologous region suggests that smg p21 has the same effector domain with the same conformation as that of ras p21s and that it shares the same effector protein(s) as ras p21s.

Another characteristic structural property of smg p21 is that it has the same consensus C-terminal sequence as ras p21s. All the small M, G proteins have unique C-terminal sequences and according to these sequences they are categorized into at least three groups. The first group has an amino acid sequence Cys-X-X-X, where X is any amino acid, and ras p21s, the rho, rac, and R-ras proteins are included in this group (1, 2, 13-15). It has been shown that ras p21s are palmitoylated at this cysteine residue and that the palmitoylation of ras p21s is essential to attach to plasma membranes and to acquire transforming activity (2). The second group has an amino acid sequence Cys-Cys, and the ypt1, rab2, and SEC4 proteins are included in this group (16-18). The third group has an amino acid sequence Cys-X-Cys, and smg p25A, smg p25B, and smg p25C are included in this group (25). smg p21 has an amino acid sequence Cys-Leu-Leu-Leu (Fig. 6). According to the C-terminal sequences, smg p21 belongs to the same group as ras p21s. We have not yet obtained evidence that smg p21 is indeed palmitoylated at this cysteine residue. However, since smg p21 is extracted from membranes by detergent and computer analysis of the hydrophobicity indicates that the protein molecule of smg p21 itself is not hydrophobic, it is most likely that smg p21 is also palmitoylated at a cysteine residue near C-terminus and attaches to membranes through this fatty acid. It is not known at present why there are three types of C-terminal sequences in small M, G proteins. However, it is tempting to speculate that different C-terminal sequences determine different intracellular compartmentalization and functions of each group of small M, G proteins. This assumption is supported by the observations that ras p21s are localized in plasma membranes (2) while the mammalian ypt1 protein is localized in the Golgi apparatus (40).

In addition to these structural properties of smg p21, smg p21 shows kinetic properties similar to those of c-Ki-ras p21 which we have recently purified from bovine brain crude membranes and characterized (27). The affinity for GTPyS of smg p21 is similar to that of c-Ki-ras p21 as shown in Table II. The time course of GTPyS-binding to smg p21 is also similar to that of c-Ki-ras p21. The turnover number of smg p21 for GTPase activity is similar to that of c-Ki-ras p21. It has been shown that GTPyS-binding to v-Ha-ras p21 is inhibited by pretreatment with NEM (41). We have previously confirmed this observation with v-Ki-ras p21 synthesized in Escherichia coli (24) and c-Ki-ras p21 purified from bovine brain (27). GTPyS-binding to smg p21 is similarly inhibited by pretreatment with NEM.

Two small M, G proteins, ARF and Gp, have thus far been purified from mammalian tissues (19-23). Although the primary structure of ARF has not been reported, smg p21 is different from ARF since smg p21 is separable from ARF by the Mono S HR5/5 column chromatography (Fig. 1), smg p21 is not recognized by the polyclonal antibody against ARF (data not shown), and ARF has been reported to lack GTPase activity (20). It is difficult to conclude the relationship between smg p21 and Gp, since Gp has been purified only partially and its properties have not been well characterized. However, it is most likely that smg p21 is different from Gp, since smg p21 is not copurified with the βγ subunits of large M, G proteins while Gp has been shown to be copurified with these subunits (22, 23).

It is well established that large M, G proteins serve as transducers for membrane receptors and regulate their specific effectors, such as adenylyl cyclase, cyclic GMP phosphodiesterase, acetylcholine-dependent K⁺ channels, voltage-dependent Ca²⁺ channels, and phosphoinositide-specific phospholipase C, in transmembrane signaling (5-8). On the other

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**TABLE II**

| Comparison of properties between smg p21 and c-Ki-ras p21 |
|-----------------------------------------------------------|
| **smg p21**  | **c-Ki-ras p21**  |
| M, \( \times 10^{-6} \) | 21 | 21 |
| Subunit structure | None | None |
| \( K_0 \) for GTPyS binding (nM) | 40 | 30 |
| Time required for full GTPyS binding (min) | 120 | 120 |
| GTPase activity (turnover number, min⁻¹) | 0.007 | 0.01 |
| Effect of NEM on GTPyS binding | Inhibition | Inhibition |

*The data are taken from Ref. 27.*

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**Fig. 9.** Time course of GTPase activity of smg p21. smg p21 (38 ng of protein) was assayed for GTPase activity with 1 μM \([γ-32P] GTP\) for various periods of time. The results shown are the representatives of three independent experiments.
hand, ras p21s have been shown to induce transformation and DNA synthesis in various cell types (2), differentiation of PC-12 cells (2), maturation of Xenopus oocytes (42), and pinocytosis in rat embryo fibroblasts (43). ras p21s have also been suggested to affect the phospholipase A2-induced arachidonic acid liberation, the phospholipase C-mediated hydrolysis of phosphoinositides, and the adenylate cyclase-mediated formation of cyclic AMP in several cell types (44–48). Moreover, in the case of SEC4 and YPT1 proteins, they have been shown to be involved in the secretory processes in yeast (18, 40). However, the functions of other small G proteins have not been clarified.

The function(s) of smg p21 is not known at present, either. However, smg p21 shows structural and kinetic properties similar to those of ras p21s among various small G proteins as discussed above. Particularly, the fact that smg p21 has the same putative effector domain as ras p21s has raised the possibility that this novel small G protein has activity(i) either similar or antagonistic to that of (those of) ras p21s described above. It is also conceivable that smg p21 with reduced GTPase activity due to the amino acid substitution in the GTPase domain has transforming activity as described for ras p21s (1, 2). The function(s) of smg p21 is now being investigated.

Acknowledgments—We are grateful to Dr. R. A. Kahn (National Institutes of Health, Bethesda, MD) for supplying us with ARF and its specific antibody and to J. Yamaguchi for skillful secretarial assistance.

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