We have determined that there are at least six GTP-binding proteins (G proteins) with M, values between 20,000 and 25,000 in the crude membrane fraction of bovine brain and have purified one of them with a M, of about 24,000 (24K G) to near homogeneity (Kikuchi, A., Yamashita, T., Kawata, M., Yamamoto, K., Ikeda, K., Tanimoto, T., and Takai, Y. (1988) J. Biol. Chem. 263, 2897-2904). In this study, we have purified another G protein with a M, of about 20,000 (20K G) to near homogeneity and have characterized it. 20K G bound maximally about 1.0 mol of [35S]guanosine 5'- (3-O-thio)triphosphate (GTPγS)/mol of protein, with a K, value of about 50 nm. [35S]GTPγS binding to 20K G was inhibited by GTP and GDP, but not by other nucleotides such as ATP, UTP, and CTP; it was also inhibited by pretreatment with N-ethylmaleimide. 20K G hydrolyzed GTP to liberate P, with a turnover number of about 0.01 min⁻¹, and was not copurified with the βγ subunits of the regulatory G proteins of adenylate cyclase. 20K G was not recognized by the antibody against the ADP-ribosylation factor for the stimulatory regulatory G protein of adenylate cyclase. Peptide map analysis showed that 20K G was not a proteolytic product of 24K G. The partial amino acid sequence of 20K G was almost identical with that deduced from the rho gene. The amino acid composition of 20K G was similar to that of the rho gene product. These results suggest that 20K G is the rho gene product and that this G protein is present in bovine brain membranes.

There is a family of G proteins with a subunit structure of αβγ which serves as transducers for membrane receptors in mammalian tissues. This family includes the stimulatory (G, ) G proteins of adenylate cyclase and transducin, which links rhodopsin to cAMP-gMP phosphodiesterase (for reviews, see Refs. 1-4). Two additional G proteins designated as G, (5, 6) and G3o (7, 8) have recently been identified and shown to belong to this family, although their definitive functions have not yet been clarified. Each G protein has a specific α, a common β, and a common γ subunit (1-6, 8). The M, values of the respective α subunits are slightly different and are between 39,000 and 52,000 (1, 2, 4-8). There are two forms of the β subunit with M, values of about 36,000 and 35,000 (1, 2, 4-6, 8, 9). M, of the γ subunit is about 10,000 (1, 2, 4-6, 8). All the α subunits have both GTP-binding and GDPase activities (1-6, 8) and amino acid sequences corresponding to the GTP-binding and GDPase domains described for elongation factor Tu (for reviews, see Refs. 1, 3, and 10). The GTP- and GDP-bound forms of the α subunits have been shown to be active and inactive, respectively, for their specific effectors (1-4).

In addition to this family of G proteins, there is another family of G proteins with M, values of about 20,000 in mammalian tissues. This family includes the G proteins of the ras, rho, and ral gene products and other novel G proteins. Three highly homologous ras genes (Ki-, Ha-, and N-ras) have been identified (11-13). The amino acid sequences deduced from the respective genes indicate that the proteins (ras p21) have M, values of about 21,000 and sequences corresponding to the GTP-binding and GDPase domains which are homologous with those found in the G proteins described above. In fact, Ki- and Ha-ras p21 produced in bacteria have been shown to possess GTP-binding and GDPase activities (14-16). Although the protein molecules of the rho and ral genes have not been identified in mammalian tissues and their properties have not been studied, these two genes are homologous with the ras genes (17-19). The amino acid sequences deduced from these genes indicate that the Aplysia rho and simian ral gene products have M, values of about 21,500 and 23,500, respectively, with sequences corresponding to the GTP- binding and GDPase domains (17-19). Recently, a novel G protein with a M, of about 21,000 designated as ARF was purified to near homogeneity from rabbit liver and bovine brain membranes (20, 21). Although the physiological function of ARF remains to be clarified, this G protein has been shown to serve as a cofactor for the cholera toxin-dependent ADP-ribosylation of G, (20, 21). A G protein with a M, of 25,000 has also been partially purified from human placenta and bovine brain membranes (22, 23). This G protein is designated as G, (22, 23). ARF and G, have been shown to be different from ras p21 (21, 22). In this paper, the G proteins with the αβγ subunit structure and those with M, values of about 20,000 are designated as large and small M, G proteins, respectively.

**Purification and Characterization of a GTP-binding Protein with a Molecular Weight of 20,000 in Bovine Brain Membranes**

**IDENTIFICATION AS THE rho GENE PRODUCT**

(Katsuhiko Yamamoto, Jun Kondo, Tadashi Hishida, Yutaka Teranishi, and Yoshimi Takai)

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(Received for publication, December 15, 1987)
In a preceding report from our laboratory (24), we separated at least six G proteins with Mr values between 20,000 and 25,000 from the crude membrane fraction of bovine brain and purified one of them with a Mr of about 24,000 (24K G) to near homogeneity. The characterization of 24K G has revealed that this G protein is distinct from ras p21, ARF, and Gi. Moreover, we have recently cloned and sequenced the cDNA of 24K G. Homology analysis of the complete amino acid sequence deduced from the cDNA of 24K G indicates that this G protein is a novel one distinct from other G proteins described previously. Extending these observations, we have purified another G protein with a Mr of 20,000 to near homogeneity from the crude membrane fraction of bovine brain and have characterized it. This G protein is designated here as 20K G. The properties of 20K G suggest that this G protein is the rgo gene product. This paper described for the first time the purification procedures and properties of the G protein which is likely to be the rgo gene product.

**EXPERIMENTAL PROCEDURES**

**Materials and Chemicals**—The crude membrane fraction was prepared from bovine brain as described previously (24). 24K G was purified to near homogeneity from the crude membrane fraction of bovine brain as described (24). A Bakerbond WP octyl column (4.6 x 250 mm) was purchased from J. T. Baker Chemical Co. Other materials and chemicals were obtained from the same sources as described previously (24).

**Purification and Characterization of 20K G**—Proteins were determined by use of the nitrocellulose filter method under the conditions specified previously (24). 20K G activity was estimated by liberation of 32Pi from [γ-32P]GTP under the conditions specified (24).

**Peptide Map Analysis**—20K G or 24K G (15 µg of each protein) was desalted on a Sephadex G-25 (PD-10) column with distilled water. Each sample was digested with Achromobacter protease I at a molar ratio of 1:300 (Achromobacter protease I:each sample) for 6 h at 37°C in 200 µl of 50 mM Tris/HCl at pH 9.0. The reaction was terminated by the addition of formic acid at a final concentration of 1%. The Achromobacter protease I-digested peptides (200 µl) were applied to a Bakerbond WP octyl column preequilibrated with 0.1% trifluoroacetic acid. The elution was performed with a 60-ml linear gradient of acetonitrile (0-60%) in a 0.1% trifluoroacetic acid at a flow rate of 1 ml/min. Peptides were detected by absorbance at 215 nm.

**Analysis of Amino Acid Sequence**—Two major peptides of 20K G separated by a Bakerbond WP octyl column were sequenced using an Applied Biosystems Model 470A automated Gas-Phase Sequencer. Phenylthiohydantoin ions were identified by high performance liquid chromatography according to the method of Tsunasawa et al. (25).

**RESULTS**

**Purification of 20K G**—G proteins were extracted with sodium cholate from the crude membrane fraction of bovine brain. The extracted G proteins were subjected to gel filtration on an Ultragel AcA-44 column, by which G proteins with Mr values between 20,000 and 25,000 were separated from large Mr G proteins, such as Gα, Gi, and Go. After small Mr G proteins were further purified by phenyl-Sepharose CL-4B column chromatography, the active fractions were subjected to hydroxylapatite column chromatography, which yielded two peaks of G proteins. The active fractions of the first peak were collected, concentrated, and then subjected to Mono Q HR5/5 column chromatography. When each fraction was assayed for GTPγS-binding activity, four peaks appeared, as shown in Fig. 1. All of these purification procedures were the same as those described previously (24), except that a YM-5 filter was used instead of a PM-10 filter for the concentration of G proteins with an Amicon ultrafiltration apparatus because ARF partly passed through a PM-10 filter.

**Purification of 20K G**

<table>
<thead>
<tr>
<th>Mr (kDa)</th>
<th>20K G</th>
</tr>
</thead>
<tbody>
<tr>
<td>20,000</td>
<td></td>
</tr>
<tr>
<td>30,000</td>
<td></td>
</tr>
<tr>
<td>40,000</td>
<td></td>
</tr>
</tbody>
</table>

**Summary of Typical Purification of 20K G**—Five GTPγS-binding peaks were separated by Mono Q HR5/5 column chromatography (24). In this experiment, the second and third peaks of this column chromatography shown in our previous paper (24) were not separated and appeared in one broad peak (second peak). This poor resolution might be due to the decrease in the quality of our Mono Q HR5/5 column.
**Table I.** When 20K G was kept frozen at -20°C, GTP-binding activity was not lost, at least for 2 weeks.

The amount of 20K G in the cholate extract from bovine brain membranes was estimated to be less than those of other well-known G proteins. About 21 µg of 20K G was purified from 858 mg of the cholate extract from the crude membrane fraction. It is difficult to calculate the exact yield of 20K G by our purification procedures since at least six small M, G proteins were separated by column chromatography. However, on the assumption that these six small M, G proteins are purified with the same yields, the yield of the purification of 20K G is calculated to be about 11%. Therefore, it could be estimated that about 0.02% of the total proteins in the cholate extract of the crude membrane fraction was 20K G. The amount of 20K G is one-fifth those of 24K G, Gα, and ARF and much less (at least 40-fold less) than those of Gα, Gα transducin, and Gα ras p21.

**Physical Properties**—When the purified preparation of 20K G was subjected to SDS-PAGE (8-16% polyacrylamide) and the protein was stained with silver after electrophoresis, only a single band was observed, as shown in Fig. 3A. The M, of 20K G was estimated to be about 20,000, as shown in Fig. 3B. In this experiment, 20K G was boiled in the presence of 2-mercaptoethanol and subjected to SDS-PAGE in this reducing agent. When the same experiment was done under non-reducing conditions, the same result was obtained (data not shown).

**[^35S]GTPγS-binding Activity**—20K G bound[^35S]GTPγS in a time-dependent manner. Fig. 4 shows the comparison of the time courses of 20K G and 24K G.[^35S]GTPγS-bound to 20K G more slowly than to 24K G. 20K G bound[^35S]GTPγS in a dose-dependent manner. Scatchard plot analysis showed that both 20K G and 24K G bound[^35S]GTPγS maximally at 1.0 ± 0.1 mol (mean ± S.E.)/mol of protein, with Ka values of 50 ± 5 and 46 ± 5 nm (mean ± S.E.), respectively. The Ka value of 20K G was 2-4-fold higher than those of Gα, Gα transducin, and Gα ras p21 (1, 2, 4, 14, 15, 28).

**Specificity for Nucleotides**—[^35S]GTPγS binding to 20K G was progressively inhibited by increasing concentrations of non-radioactive GTPγS, GTP, or GDP. Among the three nucleotides, GTPγS was 2-3-fold more active than GTP and GDP. Both GTP and GDP showed no difference in this capacity. Other nucleotides such as ATP, CTP, and UTP were not effective.

**Inhibition of[^35S]GTPγS Binding to 20K G by Pretreatment with NEM**—It has been shown that[^35S]GTPγS binding to Ha-ras p21 is inhibited by pretreatment with NEM (29). We have previously confirmed this observation with Ki-ras p21 (24).[^35S]GTPγS binding to 20K G was also inhibited by pretreatment with NEM, and this inhibition was blocked by the simultaneous presence of dithiothreitol. In contrast to ras p21 and 20K G,[^35S]GTPγS binding to 24K G was not inhibited by pretreatment with NEM, as described previously (24).

**GTPase Activity**—20K G showed GTPase activity as estimated by the liberation of ^32P from[^32P]GTP. Fig. 5 shows the time courses of 20K G and 24K G for the GTPase activity. There were lag periods for the GTPase activities of both G proteins, but the lag period for 20K G was longer than that for 24K G. This difference might be due to the fact that[^35S]GTPγS-binding to 20K G required a longer time than that to 24K G (see Fig. 4). The turnover number of 20K G was calculated to be about 0.01 ± 0.002 min^-1 (mean ± S.E.), whereas that of 24K G was about 0.04 ± 0.006 min^-1 (mean ± S.E.), as described (24). The value of 20K G is far less than those of Gα, Gα transducin, and Gα ras, but is comparable to that of c-ras p21 (1, 2, 4, 6, 16).

**Immunological Cross-reactivity of 20K G with Antibody against ARF**—We have previously shown that ARF is eluted on the first peak of Mono Q HR5/5 column chromatography (24). This result indicates that 20K G is separable from ARF by this column chromatography. In order to obtain additional

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**Table I.** Purification of 20K G

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total GTPγS-binding amount (nmol)</th>
<th>Specific activity (nmol/mg)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholate extract</td>
<td>95</td>
<td>858</td>
<td>484</td>
<td>0.56</td>
<td>100</td>
</tr>
<tr>
<td>Ulrogel Aca-44 (second peak)</td>
<td>200</td>
<td>90</td>
<td>223</td>
<td>2.5</td>
<td>46</td>
</tr>
<tr>
<td>Phenyl-Sepharose CL-4B</td>
<td>120</td>
<td>32</td>
<td>142</td>
<td>4.4</td>
<td>29</td>
</tr>
<tr>
<td>Hydroxylapatite (first peak)</td>
<td>240</td>
<td>6.8</td>
<td>51</td>
<td>7.5</td>
<td>11</td>
</tr>
<tr>
<td>Mono Q HR5/5 (third peak)</td>
<td>2.5</td>
<td>0.08^a</td>
<td>2.9</td>
<td>36</td>
<td>0.6</td>
</tr>
<tr>
<td>Re-Mono Q HR5/5 (second peak)</td>
<td>2.5</td>
<td>0.025^a</td>
<td>1.0</td>
<td>40</td>
<td>0.21</td>
</tr>
</tbody>
</table>

^a Values were determined by densitometric tracing of the gel after SDS-PAGE as described under "Experimental Procedures."
Purification and Characterization of 20K G

Fig. 3. Analysis of 20K G on SDS-PAGE. A, protein staining. 20K G (200 ng of protein) was subjected to SDS-PAGE (8-16% polyacrylamide). The protein was visualized with silver staining. B, estimation of Mr. The protein markers used were bovine serum albumin (Mr = 66,000) (point 1), glyceraldehyde-3-phosphate dehydrogenase (point 2), trypsin inhibitor (point 3), and myoglobin (Mr = 17,000) (point 4). The arrow indicates the position of 20K G.

Fig. 4. Time courses of [35S]GTPγS binding to 20K G and 24K G. [35S]GTPγS-binding activity of 20K G or 24K G (50 ng of each protein) was assayed for various periods of time. The radioactivity of [35S]GTPγS maximally bound to 20K G or 24K G was about 4000 cpm. ●, 20K G; ○, 24K G. The results shown are representative of three independent experiments.

Fig. 5. GTPase activities of 20K G and 24K G. GTPase activity of 20K G or 24K G (100 ng of each protein) was assayed with 1 μM [γ-32P]GTP for various periods of time. ●, 20K G; ○, 24K G. The results shown are representative of three independent experiments.

Fig. 6. Immunological cross-reactivity of 20K G with antibody against ARF. A, protein staining. 20K G or ARF (200 ng of each protein) was subjected to SDS-PAGE (8-16% polyacrylamide). The proteins were visualized with silver staining. Lane 1, 20K G; lane 2, ARF. B, immunoblot analysis of 20K G and ARF. 20K G or ARF (200 ng of each protein) was subjected to SDS-PAGE (8-16% polyacrylamide). After the protein on the gel was transferred to a nitrocellulose sheet, the sheet was immunoblotted with the polyclonal antibody against ARF. Lane 1, 20K G; lane 2, ARF.

the peptide maps of both proteins. Pure samples of 20K G and 24K G were digested completely by Achromobacter protease I. This protease has been shown to hydrolyze specifically lysyl bonds (30). The digested samples were separately subjected to Bakerbond WP octyl column chromatography under the same conditions. The peptides were monitored spectrophotometrically at 215 nm. The peptide peaks of 20K G did not coincide with those of 24K G at all, as shown in Fig. 7.

Partial Amino Acid Sequence and Amino Acid Composition of 20K G—The major peaks, peaks 1 and 2 shown in Fig. 7, were sequenced by use of an automatic Sequencer. The analysis of sequence homology revealed that the amino acid se-

Although the Mr of ARF was estimated to be about 21,000 by Kahn and Gilman (20, 21), it was estimated to be about 19,500 under our conditions. The exact reason for this difference is not known, but may be due to the different molecular markers used in their and our laboratories.
peaks were monitored spectrophotometrically at 215 nm. The peaks purified preparations shown in Fig.
residues 99-104 and 52-74, respectively, of the those deduced from sequences of peaks 1 and 2 were almost identical to those of the amino acid sequences of the human protein since the total amino acid composition of 20K G is similar to that of the GTP-binding domain (17). In spite of these similarities between both proteins, they have variable regions. Particularly, maximal divergence is observed near the carboxyl-terminal amino acid (17).

In this paper, we have purified 20K G to near homogeneity and characterized it. 20K G is a GTP-binding protein with a K\textsubscript{d} value for GTP\textsubscript{y}S of about 50 nM and has GTPase activity with a turnover number of about 0.01 min\textsuperscript{-1}. 20K G is found in the crude membrane fraction of bovine brain and is extracted with sodium cholate from the membranes. The partial amino acid sequence of 20K G is almost identical to those of the Aplysia and human rho gene products. Moreover, the amino acid composition of 20K G is similar to that of the Aplysia rho gene product, with the exception of a few amino acids. The content of lysine in 20K G was considerably lower than that in the Aplysia rho gene product. This difference may be due to the species difference since the Aplysia rho gene product has a high lysine content, but the human rho gene product does not, in the variable regions near the carboxyl-terminal amino acid of each protein (17). These properties of 20K G strongly suggest that this G protein is the bovine brain rho gene product, although the possibility cannot be neglected completely at present that the 20K G gene is highly homologous with, but distinct from, the rho gene until the total nucleotide sequence of the 20K G gene is determined. It has been shown (29) that a cysteine residue in the vicinity of the GTP-binding domain of Ha-ras p21 is sensitive to a thiol-specific reagent, NEM, and that pretreatment of this protein with NEM diminishes its GTP-binding activity. Similarly, the GTP\textsubscript{y}S-binding activity of 20K G is inhibited by pretreatment with NEM. This result is consistent with the

![FIG. 7. Peptide map analysis of digested peptides generated from 20K G and 24K G. 20K G or 24K G (10 pg of each protein) was digested with Achromobacter protease I. The digested protein was subjected to Bakerbond WP octyl column chromatography. Peptide peaks were monitored spectrophotometrically at 215 nm. The peaks indicated by arrows were not peptides, but CHAPS included in the purified preparations of 20K G and 24K G. A, 20K G; B, 24K G.](image-url)

**TABLE II**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>20K G*</th>
<th>Aplysia rho gene product*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>21.1</td>
<td>21</td>
</tr>
<tr>
<td>Thr</td>
<td>9.2</td>
<td>9</td>
</tr>
<tr>
<td>Ser</td>
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<tr>
<td>Glx</td>
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<tr>
<td>Pro</td>
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<tr>
<td>Ile</td>
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<tr>
<td>Leu</td>
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<td>Tyr</td>
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<td>6</td>
</tr>
<tr>
<td>Phe</td>
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<td>6</td>
</tr>
<tr>
<td>Lys</td>
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<td>His</td>
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<tr>
<td>Arg</td>
<td>11.8</td>
<td>10</td>
</tr>
<tr>
<td>Trp</td>
<td>0.3*</td>
<td>2</td>
</tr>
</tbody>
</table>

*The number of residues/molecule was calculated according to a M, of 20,000 for 20K G.

*Values were calculated from the data of Madaule and Axel (17).

*ND, not determined.

*Methionine and tryptophan were destroyed by oxidation under our conditions.

The rho gene (the ras-homologous gene) was first isolated from a cDNA library from the abdominal ganglia of Aplysia by Madaule and Axel (17). This gene was also detected in yeast, Drosophila, rat, and man (17). The rho gene is conserved, and amino acid sequences of the Aplysia and human gene products are 85% homologous (17). The Aplysia and human rho gene products share 35% amino acid homology with Ha-ras p21 (17), and the rho and ras gene products have common properties. 1) Both have similar M, values of about 20,000; 2) both have amino acid sequences corresponding to the GTP-binding and GTPase domains; 3) both have carboxyl-terminal sequences required for attachment to the inner leaflet of the cytoplasmic membranes; and 4) both have a cysteine residue in the vicinity of the GTP-binding domain (17). In spite of these similarities between both proteins, they have variable regions. Particularly, maximal divergence is observed near the carboxyl-terminal amino acid (17).

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**DISCUSSION**

The rho gene (the ras-homologous gene) was first isolated from a cDNA library from the abdominal ganglia of Aplysia by Madaule and Axel (17). This gene was also detected in yeast, Drosophila, rat, and man (17). The rho gene is conserved, and amino acid sequences of the Aplysia and human gene products are 85% homologous (17). The Aplysia and human rho gene products share 35% amino acid homology with Ha-ras p21 (17), and the rho and ras gene products have common properties. 1) Both have similar M, values of about 20,000; 2) both have amino acid sequences corresponding to the GTP-binding and GTPase domains; 3) both have carboxyl-terminal sequences required for attachment to the inner leaflet of the cytoplasmic membranes; and 4) both have a cysteine residue in the vicinity of the GTP-binding domain (17). In spite of these similarities between both proteins, they have variable regions. Particularly, maximal divergence is observed near the carboxyl-terminal amino acid (17).

In this paper, we have purified 20K G to near homogeneity and characterized it. 20K G is a GTP-binding protein with a K\textsubscript{d} value for GTP\textsubscript{y}S of about 50 nM and has GTPase activity with a turnover number of about 0.01 min\textsuperscript{-1}. 20K G is found in the crude membrane fraction of bovine brain and is extracted with sodium cholate from the membranes. The partial amino acid sequence of 20K G is almost identical to those of the Aplysia and human rho gene products. Moreover, the amino acid composition of 20K G is similar to that of the Aplysia rho gene product, with the exception of a few amino acids. The content of lysine in 20K G was considerably lower than that in the Aplysia rho gene product. This difference may be due to the species difference since the Aplysia rho gene product has a high lysine content, but the human rho gene product does not, in the variable regions near the carboxyl-terminal amino acid of each protein (17). These properties of 20K G strongly suggest that this G protein is the bovine brain rho gene product, although the possibility cannot be neglected completely at present that the 20K G gene is highly homologous with, but distinct from, the rho gene until the total nucleotide sequence of the 20K G gene is determined. It has been shown (29) that a cysteine residue in the vicinity of the GTP-binding domain of Ha-ras p21 is sensitive to a thiol-specific reagent, NEM, and that pretreatment of this protein with NEM diminishes its GTP-binding activity. Similarly, the GTP\textsubscript{y}S-binding activity of 20K G is inhibited by pretreatment with NEM. This result is consistent with the
fact that the Aplysia and human rho gene products have a cysteine residue in the vicinity of the GTP-binding domain (17). Bovine brain 20K G shows a Mr of about 20,000. This Mr is slightly different from that of the Aplysia rho gene product (Mr = 21,500). The slight difference of the Mr values between bovine 20K G and the Aplysia rho gene product may be due to the difference of amino acid sequences. During the preparation of this manuscript, Anderson and Lacal (31) purified the Aplysia rho gene product overexpressed in Escherichia coli and characterized it. The Aplysia rho protein shows GTP-binding and GTPase activities, with a K\textsubscript{m} for GTP of 303 nM and a turnover number of 0.106 min\textsuperscript{-1}, respectively. These values are quite different from those of 20K G purified from the crude membrane fraction of bovine brain in this paper. The exact reason for these differences is not clear, but may be due to the different origins or assay conditions or to differences between the proteins expressed bacterially and those purified from a mammalian tissue.

20K G shows properties similar to those of ras p21 as predicted from the structure of the rho gene product (17). 1) Both have similar Mr, values of about 20,000; 2) both have GTP-binding and GTPase activities; 3) the GTP-binding activities of both are sensitive to pretreatment with NEM; and 4) both are localized in the membranes. In spite of the similarities between 20K G and ras p21, the partial amino acid sequence of 20K G is different from that of ras p21. Moreover, c-ras p21 is mostly eluted in the second peak and is partly eluted in the first peak, whereas 20K G is eluted in the first peak by hydroxylapatite column chromatography, as described previously (24). c-ras p21 slightly contaminates the third peak (20K G) of the first Mono Q HR5/5 column chromatography, but this contaminating c-ras p21 is separated from 20K G by re-Mono Q HR5/5 column chromatography (data not shown). Moreover, several monoclonal antibodies against ras p21 are not cross-reactive with 20K G (data not shown). These results indicate that 20K G is clearly distinct from c-ras p21.

In addition to the rho gene, three ras-related genes have been identified. One is designated as the ral gene and has been identified in simian B-lymphocytes (19). The ral gene codes for a protein with a Mr of about 23,500 and shares 50% amino acid homology with the K\textsubscript{i}, H\textsubscript{a}, and N-ras gene p21s (19). The protein molecule of the ral gene has been shown to be involved in the regulation of adenylate cyclase (36). The mammalian ras and yeast RAS1 gene products have been suggested to be involved in the regulation of the phospholipase C-mediated hydrolysis of phosphoinositides (37-39). Therefore, it is conceivable that small M, G proteins are also involved in transmembrane signaling, but presumably in a manner different from that of large M, G proteins. Besides the functions of small M, G proteins in transmembrane signaling, the YPT1 gene product has been shown to be involved in the regulation of small G proteins. The yeast RAS2 gene product has been shown to be involved in the regulation of adenylate cyclase (36). The mammalian ras and yeast RAS1 gene products have been suggested to be involved in the regulation of the phospholipase C-mediated hydrolysis of phosphoinositides (37-39). Therefore, it is conceivable that small M, G proteins are also involved in transmembrane signaling, but presumably in a manner different from that of large M, G proteins. Besides the functions of small M, G proteins in transmembrane signaling, the YPT1 gene product has been shown to be involved in the regulation of microtubular arrangement (36), and the SEC4 gene product is involved in the secretory processes in yeast (34). By analogy, some mammalian small M, G proteins may also be related to cytoskeletal arrangement and the regulation of secretory processes. Investigation of the functions of small M, G proteins is essential to understand the new regulatory mechanisms of various cell functions.

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REFERENCES

Purification and Characterization of 20K G