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Microsomal and cytosolic fractions of guinea pig hepatocytes contain 100-kilodalton GTP-binding proteins reactive with antisera against α subunits of stimulatory and inhibitory heterotrimeric GTP-binding proteins

DANIEL UDRISAR AND MARTIN RODBELL

Section on Signal Transduction, Laboratory of Cellular and Molecular Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

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ABSTRACT **Guinea pig hepatocytes fractionated by differential centrifugation into plasma membrane-enriched, microsomal, and cytosolic fractions were examined for their content of α and β subunits of heterotrimeric GTP-binding proteins (G proteins) involved in signal transduction. α subunits of stimulatory (G_s) and inhibitory (G_i) proteins were detected by immunoblots with antisera reactive with the carboxyl-terminal decapeptide regions of these proteins. Unexpectedly, antisera (including immunopurified) to the α subunit but not the β subunit reacted with a band of 100-kDa proteins in both the microsomal and cytosolic fractions. The immunoreactive 100-kDa proteins are not substrates for ADP-ribosylation catalyzed by pertussis toxin, cholera toxin, or diptheria toxin. Protease digests of the 100-kDa proteins yielded immunoreactive peptides that are distinctly different from those obtained from protease digests of α subunits of heterotrimeric G proteins. The 100-kDa protein(s) reactive with antisera to G_i α subunit bind to GTP-agarose but not to ATP-agarose. It is concluded that the immunoreactive 100-kDa proteins in microsomal and cytosolic fractions are structurally distinct G proteins from those linked to receptors in the plasma membrane and other G proteins such as elongation factor 2. Conceivably, the 100-kDa proteins represent a new class of G proteins.**

GTP-specific binding proteins (G proteins) play a vital role in the regulation of a variety of cellular processes, including signal transduction via hormone receptors at the plasma membrane (heterotrimeric G proteins) (1), protein chain extension (2), tubulin polymerization (3), and the processing and trafficking of vesicles in the Golgi complex (4). The family of G proteins has grown recently to include small molecular weight proteins (21–25 kDa) that share similar sizes with oncogenic G proteins such as RAS (5).

Recent studies indicate that the G protein α subunits are not necessarily confined to the cell membrane. For example, the α subunit of the inhibitory G protein (G_i) in human neutrophils is present both in the plasma membrane and in a specific granule-enriched fraction thought to represent a reservoir of receptor-coupled G proteins for translocation to the plasma membrane (6). In rat adipocytes, α subunits of G_i and the stimulatory G protein (G_s) are found in pinosomes, the formation or processing of which are regulated by hormones or agents that stimulate cAMP formation (7). Immunocytochemical studies of neuronal and glial cells suggest that the α subunit of G_o, a G protein of unknown function, is present at the surface membrane but is also diffused over the entire glial cell and densely concentrated around the nucleus (8). In the present study, we have used antibodies raised against the carboxyl-terminal decapeptide regions of the α subunits of G_i and G_s (9) and against the β subunits to determine their distribution in various subcellular fractions obtained from guinea pig hepatocytes. As expected, the plasma membrane-enriched fraction (P fraction) was enriched in α subunits of G_i and G_s and β subunits. Unexpectedly, the microsomal fraction (M fraction) and cytosolic fractions (C fraction) displayed on immunoblots a band of 100-kDa proteins reactive with antisera against the α subunits of both G_i and G_s but not with antiserum against the β subunit. This study documents evidence that the 100-kDa band consists of at least two proteins that are structurally distinct from the typical G protein α subunits involved in receptor-mediated signal transduction at the plasma membrane.

MATERIALS AND METHODS

Materials. Materials were obtained as follows: buffers, salts, chelators, detergents, nucleotides, bovine serum albumin, dithiothreitol, 3,3′-diaminobenzidine, aprotinin, GTP-agarose, and ATP-agarose were from Sigma; *Staphylococcus aureus* V8 (SA-V8) protease was from Boehringer Mannheim; reagents and equipment for gel electrophoresis and immunoblotting were from Bio-Rad; pre-stained molecular weight standards were from Diversified Biotech; pertussis toxin (PTX), cholera toxin (CTX), and diphtheria toxin (DTX) were from List Biological Laboratories; rabbit anti-goat IgG and goat peroxidase antiperoxidase were from Organon Teknika–Cappel; [^{32}P]NAD$^+$ [specific activity, 30 Ci/mmol (1 Ci = 37 GBq)] was from DuPont-New England Nuclear; nitrocellulose paper (BA-83, 0.2 μm) was from Schleicher & Schuell; and Percoll and protein A-Sepharose were from Pharmacia.

Antisera to G Protein Subunits. Immunoblots were probed with rabbit polyclonal antisera obtained from the following sources. Allen Spiegel (National Institutes of Health, Bethesda, MD) supplied the AS/7 and AS/6 antisera (immunopurified) raised against the decapeptide Lys-Glu-Asn-Leu-Lys-Asp-Cys-Gly-Leu-Phe, which represents the carboxyl terminus of bovine transducin α and is highly conserved in α-1 and α-2 subtypes of the G_i α subunit; the AS antisera also recognize the G_i α-3 subunit; antiserum RM/1 (130R) against the α subunit of G_s was generated against the carboxyl-terminal decapeptide of that subunit (9). Antisera reactive

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Abbreviations: G proteins, GTP regulatory or binding proteins; G_i and G_s, inhibitory and stimulatory G proteins; P fraction, plasma membrane-enriched fraction; M fraction, microsomal fraction; C fraction, cytosolic (135,000 × *g* soluble) fraction; PTX, pertussis toxin; DTX, diphtheria toxin; CTX, cholera toxin; EF-2, elongation factor 2; SA-V8 protease, *Staphylococcus aureus* V8 protease; RIPA, radioimmunoprecipitation assay.

with the G protein β subunit was supplied by David Manning (University of Pennsylvania).

Preparation of Hepatocytes. Hepatocytes were isolated from male Hartley guinea pigs by a collagenase digestion procedure originally described by Berry and Friend (10), modified by Seglen (11), and used here as described by Burgess *et al.* (12). Prior to homogenization, the cells were washed with 100 ml of medium containing 10 mM Tris·HCl, pH 7.4/1 mM EDTA/0.25 M sucrose and were collected by centrifugation in a Sorvall RC-2B centrifuge at 500 × *g* for 2.5 min. This and all further procedures were carried out at 4°C.

Homogenization of Hepatocytes. The cells ($8–10 \times 10^6$) were resuspended in 3 ml of 10 mM Tes (pH 7.4), and the suspension was placed in a 50-ml Amicon ultrafiltration device. Nitrogen pressure [50 pounds per square inch (psi); 1 psi = 6.9×10^3 Pa] was applied for 10 min, followed by rapid decompression; this procedure was repeated. The cells were then homogenized in a 7-ml glass homogenizer (Kontes) with five up-and-down strokes of the plunger. Nuclei were removed by centrifugation at 500 × *g* for 2.5 min.

Preparation of P, M, and C Fractions. These fractions were prepared by the Percoll fractionation procedure described by Heyworth *et al.* (13). The postnuclear supernatant was centrifuged at 27,500 × *g* for 15 min. The supernatant fluid was saved for preparation of M and C fractions. The pellet, containing the bulk of the P fraction, was resuspended in 10 mM Tes (pH 7.4), and the suspension was centrifuged on a 13.5% Percoll gradient for 15 min at 27,500 × *g*. The top band containing the plasma membranes was washed twice by resuspension and centrifugation at 48,000 × *g* in 10 ml of the Tes buffer. The washed suspension was layered over a 2-ml cushion of 54% (wt/vol) sucrose and centrifuged at 48,000 × *g* for 15 min. The membranes found at the interface with sucrose were carefully removed with a Pasteur pipette and were washed once with Tes buffer followed by suspension in the buffer at a concentration of 5–6 mg of protein per ml. The initial supernatant was centrifuged at 135,000 × *g* for 60 min in a TL-100 Beckman ultracentrifuge. The resultant pellet (M fraction) was suspended in Tes buffer (5–10 mg of protein per ml). The three fractions (P, M, and C) were stored at −70°C in small aliquots that were thawed and used only once for the experiments.

ADP-Ribosylation by PTX, CTX, and DTX. The conditions used for ADP-ribosylation by PTX were those described by Ribeiro-Neto *et al.* (14). Briefly, P- or C-fraction membranes (each at 10 μg of protein) were incubated in 135 μl of 10 mM Tris·HCl, pH 7.5/5.3 mM thymidine/1 mM ATP/6 mM dithiothreitol/10 μCi of [^{32}P]NAD$^+$. Incubations were carried out overnight at 4°C in the presence of 5 μg of activated PTX per assay or with buffered control. The toxin was activated by incubating 50 μg of toxin per 100 μl at 30°C for 20 min with an equal volume of 100 mM dithiothreitol and 4 μl of 100 mM ATP. PTX-treated samples were solubilized and subjected to NaDodSO$_4$/PAGE (15). ADP-ribosylation of the M fraction by CTX and by DTX were carried out as described, respectively, by Tamir and Gill (16) and Carroll and Collier (17). The M fraction (50 μg of protein) was incubated with 5 μg of DTX (preactivated with 40 mM dithiothreitol) and 5 μCi of [^{32}P]NAD$^+$ at 25°C for 15 min. The sample was solubilized with radioimmunoprecipitation assay (RIPA) buffer (see below) and used as described below.

NaDodSO$_4$/PAGE, Immunoblotting, and Electroelution. Electrophoresis was carried out with minigels (7 cm × 8 cm × 1 mm) containing a 5–20% gradient of polyacrylamide (15). Samples (5–10 μg of protein) were electrophoresed for 45 min at 200 V. After electrophoresis, the gels were soaked in transfer buffer [25 mM Tris·HCl/192 mM glycine/20% (vol/vol) methanol, pH 8.3] for 10 min, sandwiched between a sheet of nitrocellulose paper and two sheets of blotting paper, and assembled into an immunoblotting apparatus (Bio-Rad), where proteins were electrotransferred to nitrocellulose paper at 70 V (350 mA) in transfer buffer. After immunodetection (18, 19), regions corresponding to immunoreactive bands were excised from the gel and electroeluted at 50 V for 14–20 hr in electroelution buffer (50 mM Tris·HCl, pH 7.4/1 mM EDTA/0.1% SDS) following established procedures (20, 21).

Epitope Mapping. The electroeluted material was mixed with sample buffer (final concentrations: 125 mM Tris·HCl, pH 6.8/0.5% NaDodSO$_4$/10% glycerol/0.0001% bromophenol blue). Samples were heated at 100°C for 2 min. Limited proteolysis was performed for 60 min at 25°C with 25 μg of SA-V8 protease per ml (22). Subsequent to NaDodSO$_4$/PAGE and transblotting, the samples were immunodetected as described above.

Immunoprecipitation. Microsomal proteins (50 μg) were solubilized on ice with RIPA buffer (150 mM NaCl/50 mM sodium phosphate, pH 7.2/2 mM EDTA/1 mM dithiothreitol/10 μg of aprotinin per ml/1.0% NaDodSO$_4$/12% sodium deoxycholate/1% Triton X-100) containing 10% (vol/vol) glycerol. Immunoprecipitation was carried out at 4°C essentially by the procedures of Sefton *et al.* (23). The solubilized material was incubated with rabbit serum (dilution with buffer, 1:63) for 1 hr, followed by treatment with protein A-Sepharose (1 mg/μl of serum) for 30 min. Nonspecific protein bound to the Sepharose was removed by centrifugation [2 min at 10,000 rpm (100,000 × *g*) in an Eppendorf microcentrifuge]. The indicated specific antiserum was added to the supernatant, and the mixture was incubated for 3 hr. The sample was again treated with protein A-Sepharose and processed as above. The supernatant was carefully removed (supernatant 1) and the precipitate (pellet 1) was washed twice with RIPA buffer, once with a solution of 150 mM NaCl/50 mM sodium phosphate, pH 7.2/2 mM EDTA, and then taken up in Laemmli buffer (15). An aliquot of the supernatant was also prepared similarly for NaDodSO$_4$/PAGE.

Binding of Cytosolic Proteins to GTP-Agarose. The cytosolic fraction (2 mg of protein per ml) was incubated for 3 hr at 4°C with 0.5 ml of a GTP-agarose suspension (0.125 ml of bed volume) in buffer A (10 mM Tris·HCl, pH 7.2/1.2 mM $MgCl_2$/0.2 mM EDTA/100 mM NaCl/1 mM dithiothreitol/10% glycerol). The mixture was separated by centrifugation in a microcentrifuge. The supernatant was added to a fresh suspension of GTP-agarose, incubated as above, and centrifuged. Both the first and second pellets were washed three times with buffer B (same as buffer A except that EDTA was 2.0 mM). All fractions were treated with Laemmli buffer and subjected to NaDodSO$_4$/PAGE; the electrophoresed material was transblotted and subsequently immunostained with AS/6 or RM/1 antisera. Identical procedures were carried out on the cytosolic fraction using ATP-agarose in the same amounts as GTP-agarose.

Other Analyses. Protein concentrations were determined according to Bradford (24) with bovine serum albumin as standard.

RESULTS

Fractionation of guinea pig hepatocytes on Percoll gradients (13) was followed as a convenient, relatively rapid means of separating homogenates into P, M, and C fractions. Immunoblotting with antisera (AS/6 or AS/7 and RM/1) obtained against the carboxyl-terminal decapeptide regions, respectively, of G_i and G_s α subunits along with the ADP-ribosylating activities of PTX and CTX were used as the primary means of detecting the presence of these proteins. Immunoblots with AS/6 or AS/7 antisera showed that the P fraction is enriched with 40-kDa proteins (Fig. 1*A*). G_i α subunits were typically ADP-ribosylated by PTX in the presence of NAD$^+$; the same 40-kDa band was labeled with

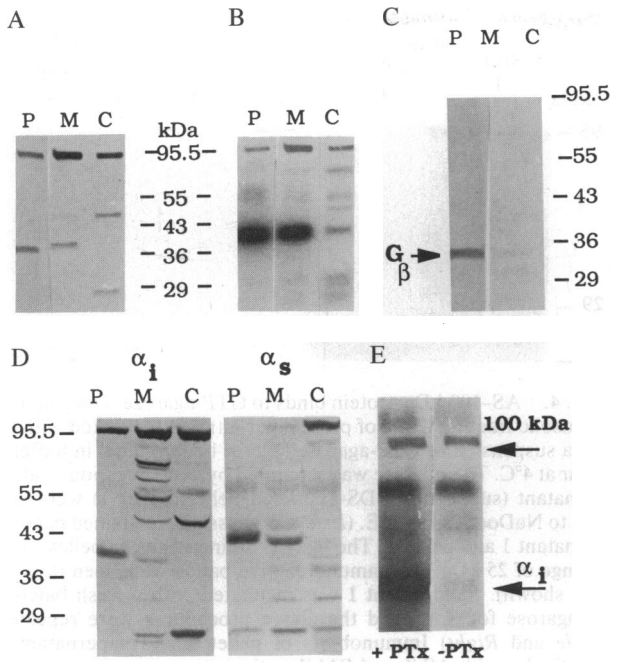

FIG. 1. Analyses of immunoreactivity and ADP-ribosylation catalyzed by PTX of P, M, and C fractions with antisera against carboxyl-terminal decapeptides of the α subunits of G_i (α_i; antibodies AS/6 or AS/7) and G_s (α_s; antibody RM/1) and the β subunit of G proteins (G_β). The subcellular fractions were obtained from guinea pig hepatocytes and were analyzed for PTX-catalyzed [^{32}P]ADP-ribosylation and immunoreactivity as described. (*A*) Immunoblots of P, M, and C fractions. Protein (5 μg) from each fraction was subjected to NaDodSO$_4$/PAGE and then transblotted and immunodetected with antibody AS/6 (dilution 1:5000). (*B*) Overlay of immunoblots in *A* with autoradiograms of the same fractions treated with [^{32}P]NAD$^+$ and PTX. (*C*) Immunoblots with anti-G_β (dilution 1:5000) obtained from NaDodSO$_4$/PAGE of 10 μg of protein per membrane fraction. (*D*) Immunoblots obtained with antibody AS/7 (against α_i) and RM/1 (against α_s) after NaDodSO$_4$/PAGE (10 μg of protein per fraction). (*E*) Overlay of immunoblots obtained with antiserum AS/6 and the autoradiogram obtained from incubating M-fraction membranes (10 μg of protein) with [^{32}P]NAD$^+$ in the presence (+) or absence (−) of PTX (for conditions, see *Materials and Methods*).

[^{32}P]NAD$^+$ when the P fraction was incubated with PTX (Fig. 1*B*). Immunoreactive and [^{32}P]ADP-ribosylated material was also found at 40 kDa in the M fraction, but in varying amounts relative to that in the P fraction possibly because of varying contamination of the M fraction with P fraction. β subunits of G proteins were also enriched in the P fraction (Fig. 1*C*), suggesting that the heterotrimeric forms of G proteins are primarily in the plasma membrane, in keeping with their role in receptor-mediated signal transduction.

Unexpectedly, antisera AS/6, AS/7, and RM/1 also reacted with material migrating at a molecular mass of about 100 kDa (Fig. 1*D*). Unlike the immunoreactive 40-kDa band, the 100-kDa material reactive with AS/6 or AS/7, referred to as AS/6–100 kDa and AS/7–100 kDa, was most prevalent in the M fraction and to a lesser extent in the C fraction (soluble fraction at 135,000 × *g*). The 100-kDa material reactive with RM/1, referred to as RM/1–100 kDa, was observed predominantly in the C fraction. None of the 100-kDa proteins was susceptible to ADP-ribosylation by PTX (Fig. 1*E*) or by CTX (not shown) under conditions that caused marked labeling of the appropriate G_i or G_s immunoreactive bands in the P fraction. The relative degree to which the AS– and RM–100-kDa immunoreactive proteins were present in the M and C fractions may in part be related to endogenous proteases in the two fractions, as illustrated by the number of immunoreactive bands (Fig. 1*D*) found in some preparations of M and C fractions. Note the markedly different patterns of immunoreactive peptides given with AS/6 and RM/1 antisera in M and C fractions. Along with the epitope-mapping studies described below, these findings suggested the presence of at least two different proteins in M and C fractions that share similar epitopes with G_i/G_s α proteins but otherwise differ in their structure and cellular distribution.

We considered the possibility that the antisera may be contaminated with antibodies against the 100-kDa proteins. This seems highly unlikely, however, since AS/6 antiserum was immunopurified by using Sepharose columns linked to the same carboxyl-terminal decapeptide used for raising both AS/6 and AS/7 antisera. Moreover, AS/6 antiserum purified by affinity chromatography also reacted with the 100-kDa proteins at titers 1/5000th that of the original antiserum (data not shown).

Epitope Mapping. Limited protease digestion with SA-V8 protease was used to compare the structures of the 100-kDa proteins and the α subunits of G proteins. In these experiments, the 100-kDa bands from the M and C fractions and the 40- to 45-kDa bands from the P fraction were excised from the gels after PAGE, electroeluted, and then subjected to protease digestion for various periods of time up to 1 hr. The digests were electrophoresed, transblotted, and immunostained with either the AS or RM antisera (Fig. 2). Immunoblots with AS/6 of the 40-kDa P-fraction digests showed a major immunoreactive band at 40 kDa after 60 min of digestion with the protease. By contrast, it is seen that AS–100-kDa material in both the M and C fractions yielded several immunoreactive bands, the smallest being a 15-kDa band. This is the expected result only if the epitopic regions in the AS–100-kDa proteins are not confined to the carboxyl-terminal portion of the protein (see *Discussion*). Similar epitope mapping of the RM–100-kDa material present in the C fraction revealed two major immunoreactive bands (36 kDa and 14 kDa) after 1 hr of protease digestion; the bands clearly differed from the pattern of immunoreactive peptides given by digests of the immunoreactive band at 45 kDa (putatively, the α subunit of G_s). The latter yielded only a single immunoreactive band at 16 kDa. The major conclusion from these epitope-mapping studies is that G_i and G_s α subunits differ in

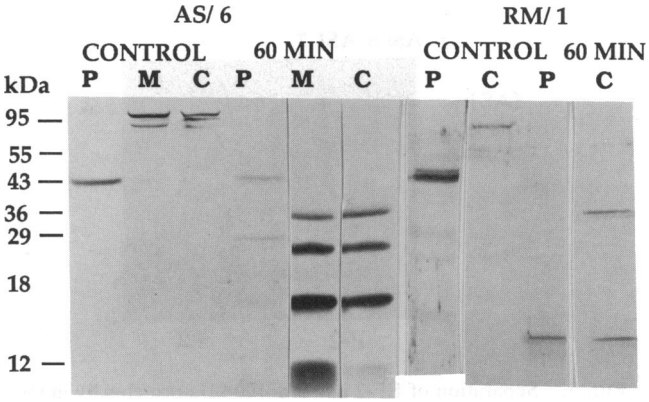

FIG. 2. Epitope mapping of hydrolysates of AS/6-immunoreactive proteins treated with SA-V8 protease. Samples (200 μg of protein) of P, M, and C fractions were loaded onto three different gradient slab gels (4–20% acrylamide) and electrophoresed. Bands corresponding to the 40-kDa material from P fraction and the 95- to 100-kDa bands from M and C fractions were excised and electroeluted (21, 22). Aliquots of the electroeluted material were incubated at room temperature with SA-V8 (25 μg/ml) for 0 and 60 min. Reactions were stopped by addition of Laemmli buffer, and the samples were subjected to NaDodSO$_4$/PAGE (15). After transblotting to nitrocellulose, the samples were incubated with either AS/6 or RM/1 antiserum and immunostained as described.

their epitope distribution from each other and from those given by AS–100- and RM–100-kDa immunoreactive proteins.

AS–100-kDa Protein Is Not Elongation Factor 2 (EF-2). A possible candidate for at least one of the immunoreactive proteins at 100 kDa is EF-2, a 100-kDa G protein involved in protein synthesis at the ribosomal level (2, 25). EF-2 is ADP-ribosylated by DTX (17). This property was used to test whether the AS–100-kDa protein band in M fraction is EF-2. Treatment of the M fraction with DTX and [^{32}P]NAD$^+$ resulted, as expected, in a radioactive band coincident with the AS–100-kDa band (Fig. 3). Immunoprecipitation of labeled M-fraction extracts with either AS/6 or AS/7 antisera resulted in a pellet that contained the immunoreactive AS–100-kDa material but none of the labeled EF-2; the latter remained in the supernatant. Hence, EF-2 is not responsible for the immunoreactivity of the AS–100-kDa fraction.

AS–100 kDa Reacts with GTP-Agarose. Because the 100-kDa proteins share epitopes with the GTP-binding subunits in G_s and G_i, it seemed possible that they may also share the common GTP-binding regions demonstrated for many of the known G proteins (26). Antisera raised against such regions did not show significant reactivity with the 100-kDa proteins on immunoblots (not shown); however, the same antiserum proved only weakly reactive with the G protein α subunits in the P fraction. Therefore, we resorted to evaluating the ability of the AS–100-kDa and RM–100-kDa proteins to interact with GTP-agarose, using essentially the method used for affinity-purifying the α subunits of G proteins (27). To avoid the possible interference of detergents on binding, only the soluble fractions were evaluated. GTP-agarose incubated with the C fraction for 3 hr absorbed proteins that were readily detected with Coomassie blue at 21–25 kDa (Fig. 4). These were specific G proteins, since they failed to adsorb to ATP-agarose (data not shown). No immunoreactive proteins representing AS–100-kDa or RM–100-kDa proteins were absorbed to GTP-agarose in the first incubation. In view of the high concentrations of 21- to 25-kDa G proteins present in the C fraction and the possibility that they may compete with other G proteins for binding to GTP-agarose, the supernatant fluid from the first incubation was incubated with a fresh batch of GTP-agarose. Extraction of the second gel revealed AS–100-kDa material; it was estimated that about

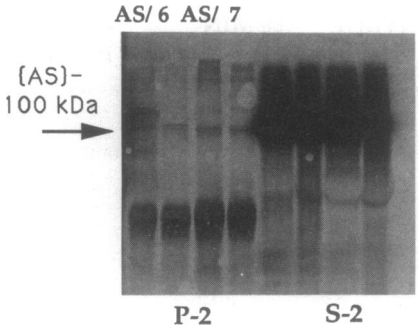

FIG. 3. Separation of EF-2 and AS–100-kDa proteins by immunoprecipitation with AS/6 and AS/7 antisera. The microsomal fraction (50 μg of protein) was incubated at room temperature for 15 min with DTX (10 μg/ml) and [^{32}P]NAD$^+$ (5 μCi). The labeled membranes were extracted with RIPA buffer and incubated with normal rabbit serum, followed by addition of protein A-Sepharose. Nonspecific immunoreactive material was removed by centrifugation. The supernatant fluid was incubated with AS/6 or AS/7 antiserum for 3 hr, followed by addition of protein A-Sepharose. The immunoprecipitate and the corresponding supernatant fluid were subjected to NaDodSO$_4$/PAGE, transblotted, and immunostained with AS/6 and AS/7 antisera. Shown is an overlay of immunoblots and the autoradiogram of immunoprecipitate (pellet P-2) and corresponding supernatant fluid (S-2).

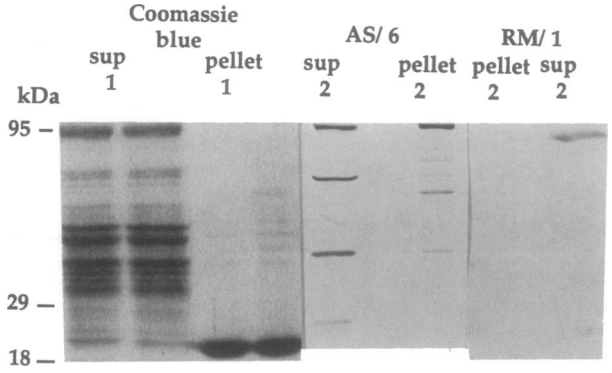

FIG. 4. AS–100-kDa protein binds to GTP-agarose. One milliliter of cytosolic fraction (2 mg of protein per ml) was incubated with 0.5 ml of a suspension of GTP-agarose (125-μl bed volume) in buffer A for 3 hr at 4°C. The mixture was separated by centrifugation, and the supernatant (sup 1) and SDS-extracted pellet (pellet 1) were subjected to NaDodSO$_4$/PAGE. (*Left*) Coomassie blue-stained bands in supernatant 1 and pellet 1. The heavily stained band in pellet 1 is in the range of 25 kDa. No immunoreactive bands were seen in pellet 1 (not shown). Supernatant 1 was incubated with a fresh batch of GTP-agarose for 3 hr, and the above procedures were repeated. (*Middle* and *Right*) Immunoblots of pellet 2 and supernatant 2, respectively, with AS/6 and RM/1 antisera.

40% of the material originally present in the C fraction was absorbed to GTP-agarose. However, RM–100-kDa material was not detected in GTP-agarose extracts of the second incubation. In corresponding studies with ATP-agarose gels, none of the immunogenic material in the 100-kDa C fraction band was detected in extracts of the gels (not shown). Hence, one or more of the immunoreactive proteins at 100 kDa specifically binds GTP.

DISCUSSION

This study shows that the M and the C fractions in guinea pig hepatocytes contain at least two proteins approximating 100 kDa that share antigenic determinants or epitopes with α subunits of G proteins (G_i and G_s) that characteristically are involved in signal transduction at the plasma membrane. The M and C immunoreactive proteins share nothing else with the α subunits of G proteins that suggest that they are structurally and functionally related. In addition to molecular size, the major differences seen for the 100-kDa proteins are: cellular distribution (enriched in M and C fractions); failure of PTX and CTX to catalyze ADP-ribosylation; different patterns of immunoreactive peptides upon protease digestion; and β subunits of G proteins, which characteristically interact with α subunits of G proteins and are involved in signal transduction at the plasma membrane (1), being rich in the P fraction, as expected but not detectable on immunoblots in the M and C fractions.

The epitope mapping studies with SA-V8 protease were particularly instructive with regard to the possible location of the antigenic determinants in AS–100-kDa and RM–100-kDa proteins. These antibodies specifically react with the carboxyl-terminal regions of G_i and G_s α proteins. SA-V8 protease cleaves proteins at glutamic and aspartic acid residues. Hence, if the antigenic determinants in the G protein α subunits and 100-kDa proteins contain the same positioning of glutamate and aspartate residues and are confined to the carboxyl-terminal regions, SA-V8 digestion should yield the same immunoreactive peptide fragments. Digestion of the 40-kDa proteins yielded diminished amounts of the protein but no other significant bands. This finding is in accord with the terminal decapeptide containing two acidic residues; the cleavage of both has a high probability of producing a loss in immunogenicity of the remaining protein and no significant

reaction of the small fragments with AS/6 antibodies. By contrast, digestion of AS–100-kDa protein yielded several immunoreactive peptides. It is likely, therefore, that the epitope is located at regions in AS–100 kDa that are not present or are not confined to the carboxyl-terminal region. The decapeptide carboxyl-terminal region of G_s α protein contains a single glutamate residue (third position from COOH end); protease digestion of G_s α protein yielded one immunogenic band at 15 kDa. This finding can be explained if the glutamate residue is not in an essential region of antigenicity. Digestion of RM–100 kDa under the same conditions yielded two major immunogenic bands (35 kDa and 14 kDa), indicating, as in the case of AS–100 kDa, that the antigenic determinants are not confined to the carboxyl-terminal region of this protein (compare Fig. 3).

Based on specific adsorption to GTP-agarose, AS–100 kDa is a G protein. However, RM–100 kDa did not bind to GTP-agarose either because it is not a G protein or its affinity for GTP is very low under the incubation conditions used for binding to GTP-agarose. In this regard, an interesting outgrowth of this study was the finding that the cytosolic fraction used in the binding study (*i*) is rich in 21- to 25-kDa G proteins (28) that bind avidly to GTP-agarose and (*ii*) effectively prevents the binding of AS–100-kDa protein(s). This finding argues for caution in interpreting the lack of binding of suspected G proteins to GTP-agarose.

AS–100 kDa has some characteristics that are strikingly similar to that of EF-2, a G protein of similar molecular weight (96 kDa) involved in protein chain elongation (2, 25). Both proteins are similarly distributed between the M and C fractions in liver (not shown). Association of EF-2 with the M fraction is not surprising in view of the interactions of this protein with ribosomes and membrane structures involved in the extension of protein chains. However, although they share similar molecular sizes and are both G proteins, AS–100 kDa and EF-2 differ in at least two respects: EF-2 is susceptible to ADP-ribosylation by DTX and is not immunoreactive with AS/6 or AS/7 antisera.

Although the positioning of the antigenic determinants in the 100-kDa proteins differs from the G protein α subunits, the fact that they share similar if not identical epitopes raises the interesting possibility that these regions are conserved in different proteins because they confer important biological functions on both classes of proteins. The carboxyl-terminal regions of G_i- and G_s α proteins are thought to be important for interactions with effectors such as adenylyl cyclase and with hormone receptors (29, 30); this region is not involved in the binding of β/γ complexes with α proteins (31, 32). The carboxyl-terminal 25 amino acid residues in G_i α subunits and transducin also show impressive homology with arrestin, a retinal protein that binds specifically to rhodopsin and that prevents binding and activation of transducin (33). However, it must be emphasized that the 100-kDa proteins are observed primarily in the M and C fractions and not in the P fraction, where most receptors are localized. Therefore, it is very unlikely that these proteins are linked to surface receptors.

There is abundant evidence that G proteins regulate a number of processes within cells that are not directly involved in receptor-mediated processes at the plasma membrane. These GTP-regulated processes include: processing or translocation of vesicles in the Golgi complex (4), sorting of proteins along secretory pathways (34); and modulation of intracellular calcium release (35). Given the apparent enrichment of AS–100-kDa protein(s) in the M fraction, involvement in one or more of the above processes is a reasonable possibility.

As a final comment, the present study suggests that immunocytochemical data obtained with the same antisera used here against G protein α subunits may not be sufficiently specific to demonstrate uniquely the presence and localization of these subunits in cells or tissues.

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