The Interferon-induced 67-kDa Guanylate-binding Protein (hGBP1) Is a GTPase That Converts GTP to GMP*

(Received for publication, October 28, 1993, and in revised form, December 27, 1993)

Martin Schwemmle‡ and Peter Staeheli§
From the Abteilung Virologie, Institut für Mikrobiologie und Hygiene, University of Freiburg, 79008 Freiburg, Germany

hGBP1 is an interferon-induced 67-kDa protein of human cells that readily binds to agarose-immobilized GTP, GDP, and GMP but not to other nucleotides. We cloned hGBP1 cDNA into a histidine-tagging vector, produced recombinant hGBP1 with 6 extra histidine residues at its N terminus in Escherichia coli, and purified this protein to near homogeneity from bacterial lysates. Purified hGBP1 hydrolyzed radiolabeled GTP but failed to hydrolyze ATP, UTP, or CTP at significant rates. Unexpectedly, the principal product of the GTP hydrolysis reaction was GDP rather than GTP. Although significant amounts of GDP were produced when the reaction was performed at 15 °C, GDP could not serve as substrate or as inhibitor of hGBP1. hGBP1 lacked guanylate cyclase and guanylpyrophosphatase activity. Degradation of GTP to GDP most likely occurred via two consecutive cleavages of single phosphate groups, because pyrophosphate was not a reaction product, and because hGBP1 failed to hydrolyze GTPyS. In vitro modification assays with radiolabeled mevalonic acid and farnesyl pyrophosphate showed that the CaaX motif at the C terminus of hGBP1 functions as an isoprenylation signal. Thus, hGBP1 is a GTPase with novel biochemical properties that may be membrane-associated in eukaryotic cells.

GTPases serve many different cellular functions; they play key roles in such basic processes as signal transduction, vesicle transport, and traaslation (1, 2). Most GTPases contain the tripartite GTP-binding consensus motif GXXGXXS, DXG, and N/TAXPG (3). From the structural analysis of p21 ras (8-10) and heterotrimeric G proteins (11), elongation factor Tu (5,6), and NrrXPG (3), it became clear that these sequences form part of the GTP binding pocket. The typical GTPases can assume two distinct conformations, a GTP-bound (active) and a GDP-bound (inactive) conformation; they thus have the potential ability to function as molecular switches (1, 7). Some GTPases, like p21ras (8-10) and heterotrimeric G proteins (11), have a CaaX sequence motif at their C termini, which functions as an isoprenylation signal (12) and thus ensures the proper anchoring of these proteins in cell membranes.

Cells treated with interferons (IFNs) respond to this stimulus by producing a set of proteins that are believed to serve as the intracellular mediators of the various effects of these cytokines (13-15). Two families of IFN-induced proteins have GTP binding activity. The Mx proteins, which mediate some of the antiviral effects of IFN, are GTPases that efficiently hydrolyze GTP to GDP (16-19). The IFN-induced guanylate-binding proteins (GBPs), whose physiological functions are unknown, can bind to agarose-immobilized guanine nucleotides (20-22). The most unusual property of GBPs is that binding to GMP and GDP occurs with similar efficiency. cDNA cloning made clear that two distinct IFN-α and IFN-γ-inducible genes code for the two human GBPs, designated hGBP1 and hGBP2 (23). Sequence analysis revealed that human and mouse GBPs contain only the first two elements of the typical GTP-binding consensus motif and that they contain a CaaX motif at their C termini (23).

Here, we show that hGBP1, the human 67-kDa guanylate-binding protein, is a GTPase that converts GTP to GDP. Since GTP analogs with a cleavage-resistant bond between the β and γ phosphates could not be hydrolyzed by hGBP1, and pyrophosphate was not a reaction product, hGBP1 seems to degrade GTP by two consecutive cleavages of single phosphate groups. We further show that hGBP1 can be isoprenylated in vitro, suggesting that it is a membrane-associated protein.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—The cDNA encoding hGBP1 was cloned into the Escherichia coli expression vector pQE9 (Diagen, Hilden, Germany) using PCR techniques. The oligonucleotide primer 1, 5′-GGCCAGCGATCCGCATCAGAGATCCACATGACAGG-3′, which has a restriction site for BamHI and corresponds to positions 72-94 of hGBP1 cDNA, and primer 2, 5′-CTGCAGGTCGACTAGGGGTGACAGGAAGGCTCTGG-3′, which has a SalI restriction site and corresponds to positions 1850-1873 of hGBP1 cDNA, were used to amplify the coding sequence of hGBP1 (23). The amplification product was digested with SalI and BamHI and cloned into the corresponding sites of pQE9. The resulting plasmid, pHis-hGBP1, codes for a fusion protein between the polypeptide Met-Arg-Glu-Ser-(His)6-Gly-Ser and the hGBP1 sequence from position 2-569. The 6 histidine residues near the N terminus are important for the purification of this protein with Ni-chelate-agarose (Diagen, Hilden, Germany). pHis-hGBP1 (C5895), which codes for the hGBP1 mutant C589S with a cysteine to serine replacement at position 589, was constructed as follows. In a first round of PCR, the complete coding region of hGBP1 was amplified with primer 1 and the oligonucleotide 5′-CTTTAGCTTATGGTACTTGCC-3′, which corresponds to positions 1849-1821 of hGBP1 cDNA (23). The resulting product had a TGC restriction site and corresponded to positions 933-2881 of hGBP1 cDNA, were used to amplify the coding sequence of hGBP1 (23). The amplification product was digested with SalI and BamHI and cloned into the corresponding sites of pQE9. The resulting plasmid, pHis-hGBP1 (C5895), which codes for the hGBP1 mutant C589S with a cysteine to serine replacement at position 589, was constructed as follows. In a first round of PCR, the complete coding region of hGBP1 was amplified with primer 1 and the oligonucleotide 5′-CTTTAGCTTATGGTACTTGCC-3′, which corresponds to positions 1849-1821 of hGBP1 cDNA (23). The resulting product had a TGC restriction site and corresponded to positions 933-2881, and the purified amplification product of the first PCR. The resulting amplification product was digested with BamHI and SalI and cloned into the corresponding sites of pQE9.

Purification of hGBP1 from E. coli—The purification of histidine-tagged hGBP1 from E. coli lysates with Ni-chelate-agarose was carried out essentially as previously described for Mx proteins (16). Briefly, pHis-hGBP1 was used to transform E. coli strain M15, and expression of histidine-tagged hGBP1 was induced by adding 0.03 mM isopropyl-β-D-
hGBP1-associated GTPase

1-thio-β,γ-galactopyranoside to the medium the culture had reached an A₆₀₀ of 0.3. At 2 h post-induction, the cells from a 1-liter culture were harvested by centrifugation and resuspended in 10 ml of ice-cold buffer A (50 mM Tris-HCl, pH 8.0, 500 mM NaCl, 5 mM MgCl₂, 10 mM 2-mercaptoethanol, 10% glycerol, 0.1% Nonidet P-40, 20 mM imidazole, and 20 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μM benzamidine, 1 mM phenylmethylsulfonyl fluoride). After sonication, the soluble proteins were separated from the cell debris by centrifugation for 30 min at 20,000 g. The supernatant was applied to a Ni-agarose column (0.5 ml bed volume) that was equilibrated with buffer A. The column was washed with 20 bed volumes of buffer A containing 20 mM imidazole and then with 20 bed volumes of buffer B (20 mM Tris-HCl, pH 8.0, 100 mM KCl, 5 mM MgCl₂, 10 mM 2-mercaptoethanol, 20% glycerol, 0.1% Nonidet P-40, 20 mM imidazole, and protease inhibitors). hGBP1 was finally eluted from the column with buffer B containing 250 mM imidazole; hGBP1 purified in this manner was typically about 95% pure.

To obtain hGBP1 of higher purity, the Ni-agarose eluate was applied to a MonoQ fast protein liquid chromatography column (HR5/5, Pharmacia, Freiburg, Germany) equilibrated with buffer C (20 mM Tris-HCl, pH 8.0, 5 mM MgCl₂, 1% glycerol, 100 mM KCl, 1 mM 2-mercaptoethanol, and 0.1% Nonidet P-40). Approximately 80% of hGBP1 was purified in this manner. Gradient elution was performed with a 20-ml linear gradient of 0.1 M KCl in buffer D at a flow rate of 0.5 ml/min. hGBP1 bound to the column under these conditions and was eluted with a 20-ml linear gradient of 0.1 M KCl in buffer D. The purity of the protein sample was determined by PAGE and Coomassie Blue staining. hGBP1 of very high purity (Fig. 1C) was obtained by this purification method.

To determine the purity of the recombinant hGBP1, the eluates were run on SDS-PAGE, and visualized by Coomassie Blue staining. The yields of purified hGBP1 were about 1–3 mg/liter of E. coli culture. hGBP1 of very high purity (Fig. 1B) was obtained after two rounds of MonoQ fast protein liquid chromatography as described under "Experimental Procedures.

First evidence that the purified recombinant hGBP1 was active came from studies with agarose-immobilized nucleotides. Samples of 5 μg of purified hGBP1 were incubated on ice with various nucleotide-agarose affinity matrices, and the bound proteins were eluted with SDS after extensive washing of the agarose beads and analyzed by SDS-PAGE and Coomassie Blue staining. Approximately 10–20% of the starting material bound to GTP, GDP, and GMP-agarose, whereas less than 10% of the material bound to CMP- or UMP-agarose (Fig. 2A). The nucleotide binding specificities of recombinant and natural hGBP1 (20, 23) thus seemed to be identical.

hGBP1 Hydrolyzes GTP to GDP—To further characterize the biochemical properties of hGBP1, we intended to analyze its unusual guanylate binding properties in more detail by filter binding assays. However, contrary to our expectations from the results of the nucleotide-agarose-binding experiments, we had suggested strong binding affinities of hGBP1 for GTP, GDP,
and GMP, we observed only weak and unstable interactions between hGBP1 and radiolabeled GTP under these conditions (data not shown).

To investigate whether hGBP1 exhibited GTPase activity, we incubated samples of affinity-purified hGBP1 with radiolabeled GTP at 37 °C. Nucleotide analysis by PEI thin layer chromatography revealed that hGBP1 converted GTP to GDP and GMP, with GMP being the predominant product (Fig. 2B). Analysis by HPLC showed that the reaction product contained no cGMP (data not shown). The GTPase activity in our hGBP1 preparations was not due to contaminating proteins from *E. coli* because other histidine-tagged proteins like dihydrofolate reductase and certain Mx mutants that we purified under identical conditions did not hydrolyze GTP (16). Furthermore, highly purified hGBP1 (Fig. 1B) was still able to convert GTP to GDP and GMP at a similar rate (Fig. 2C). The observed GTPase activity most likely reflected a true biochemical activity of hGBP1, because the product of a second independent PCR clone also hydrolyzed GTP to GDP and GMP. The specific GTPase activities of our hGBP1 preparations were typically about 50 nmol/min/mg. The hydrolysis reaction showed a pH optimum of 8.0. Addition of up to 100 mM of NaCl had no significant effect on the GTP hydrolysis rate and did not change the product composition (data not shown). By determining the relative GTPase activity of hGBP1 in the presence of different GTP concentrations, we calculated $K_m$ and $V_{max}$ values of 470 μM and 6 min$^{-1}$, respectively (Fig. 3).

To clarify the nucleotide specificity of hGBP1, we tried to inhibit the GTPase reaction with various unlabeled nucleotides, which were used at a 15-fold molar excess over GTP. GMP efficiently blocked the GTPase activity of hGBP1 (Fig. 4, lane 4), whereas GDP had no influence on the hydrolysis of GTP...
K, respectively.

GTPase assays were done with different nucleotide concentrations and 0.025 μg/μl of purified hGBP1. The 1/v versus 1/[S] plot is shown. The \( K_m \) and \( V_{max} \) values were calculated to be 470 μM and 6 min⁻¹, respectively.

![GTPase activity of hGBP1 in the presence of different nucleotides.](image)

**Fig. 3. Kinetics of hGBP1-associated GTPase activity at 37 °C.** GTPase assays were done with different nucleotide concentrations and 0.0125 μg/μl of purified hGBP1 (data not shown). The 1/v versus 1/[S] plot is shown. The \( K_m \) and \( V_{max} \) values were calculated to be 470 μM and 6 min⁻¹, respectively.

\begin{align*}
\text{Competition:} & \quad \text{GTP} \quad \text{GDP} \quad \text{GMP} \quad \text{UTP} \quad \text{ATP} \quad \text{CTP} \\
\text{Position:} & \quad \text{GMP} \quad \text{GDP} \quad \text{GTP}
\end{align*}

**Fig. 4. GTPase activity of hGBP1 in the presence of different nucleotides.** GTPase assays were performed with 0.0125 μg/μl of purified hGBP1, 12 nM [γ-32P]GTP, and a 15-fold molar excess of the indicated unlabeled nucleotides. The reaction products were analyzed by separation on PEI thin layer chromatography plates and by autoradiography. The positions of GTP, GDP, and GMP are indicated.

(Fig. 4, lane 3). This result was unexpected in light of the fact that GDP was a minor reaction product and that hGBP1 bound to GDP-agarose. ATP, CTP, and UTP failed to inhibit the hGBP1-associated GTPase activity (Fig. 4, lanes 5–7). Similarly, cGMP had no inhibitory effect (data not shown). hGBP1 failed to hydrolyze radiolabeled ATP, CTP, and UTP at a significant rate (data not shown).

**GDP Is Not a Substrate for hGBP1 but Is the Predominant GTP Hydrolysis Product at Low Temperature**—Although GDP could not inhibit the GTPase activity of hGBP1, it was detected as a minor product of the hGBP1-catalyzed GTP hydrolysis reaction (Figs. 2 and 4). We therefore tested whether GDP could serve as a substrate for hGBP1. Samples of hGBP1 were incubated with unlabeled GDP, and the reaction products were analyzed by HPLC. hGBP1 failed to hydrolyze GDP (Fig. 5, A and B), whereas GTP was readily hydrolyzed (Fig. 5, C and D).

During our attempts to define the temperature optimum for the hGBP1-associated GTPase activity, which was found to be 37 °C (data not shown), we observed that the relative amounts of newly formed GDP were higher when the GTP hydrolysis reaction was performed at lower temperature. Quantification of the products of a GTP hydrolysis reaction carried out at 15 °C revealed that GDP was indeed the predominant product; about 56% of the GTP substrate was converted to GDP (Fig. 6B). In contrast, at 37 °C only about 15% of the GTP substrate was converted to GDP; the other 85% was hydrolyzed to GMP (Fig. 6A). Temperature shift-up experiments in which the GTPase reaction temperature was raised from 15 to 37 °C confirmed our result that GDP cannot serve as a substrate; the GDP that was formed during the incubation at 15 °C was not converted to GMP after the temperature shift-up, whereas the remaining GTP was (data not shown). We further found that prolonged incubation at 15 °C of active hGBP1 (0.8 μg in 30 μl) with 200 μM GDP did not result in the formation of detectable amounts of GMP (data not shown). Taken together, these results suggested that GDP is a dead-end product of the hGBP1-catalyzed hydrolysis of GTP.

**hGBP1 Exhibits No Guanylyltransferase Activity**—A characteristic feature of guanylyltransferases is that they transiently form covalent bonds with guanylates before transferring them to target molecules (26). To examine whether hGBP1 has guanylyltransferase activity, we incubated samples of purified hGBP1 with [α-32P]GTP, precipitated the proteins with trichloroacetic acid, and analyzed them by SDS-PAGE and autoradiography. Purified vaccinia virus-derived guanylyltransferase served as a positive control. As expected, we observed strong labeling of the vaccinia virus guanylyltransferase under these conditions. However, we failed to detect significant labeling of hGBP1 (data not shown), suggesting that it has no guanylyltransferase activity.

**hGBP1-catalyzed Hydrolysis of GTP Yields No Pyrophosphate**—The finding that GTP was converted to GDP raised the possibility that pyrophosphate was a product of the hGBP1-catalyzed hydrolysis reaction. We therefore measured the pyrophosphate contents in the reaction mixtures by paper chromatography. Standard hydrolysis reactions were performed at 37 °C with highly purified hGBP1 and [γ-32P]GTP, and the products were separated on Whatman 3MM paper and visualized by autoradiography. The radiolabeled products comigrated with the monophosphate standard; no signal was observed at the position of the pyrophosphate marker (Fig. 7, lanes 4 and 8). It was possible that pyrophosphate was indeed formed but that it was rapidly degraded by contaminating pyrophosphatases. If this was the case, we would expect to see at least a transient accumulation of radiolabeled pyrophosphate, which we did not (Fig. 7). To increase the sensitivity of the assay, we added various concentrations of unlabeled pyrophosphate to the reactions. We reasoned that these additives should decrease the rate by which a contaminating pyrophosphatase will degrade the newly formed radiolabeled pyrophosphate. However, we failed to detect radiolabeled pyrophosphate under these conditions (Fig. 7). From this experiment, we concluded that our hGBP1 preparations did not contain significant amounts of pyrophosphatases or else contained so much of it that a 100-μM solution of pyrophosphate got degraded within 5 min. Further control experiments with unlabeled pyrophosphate showed that the latter possibility was incorrect (data not shown). Taken together, these results suggested that pyrophosphate was not a product of the hGBP1-catalyzed hydrolysis of GTP.

**hGBP1 Fails to Hydrolyze GTP Analogs with a Cleavage-Resistant Bond between the β and γ Phosphates**—If hydrolysis of GTP to GMP by hGBP1 indeed occurred via two consecutive cleavages of single phosphate groups rather than by a single cleavage of pyrophosphate, one would expect that hGBP1 fails to hydrolyze GTP analogs that cannot be cleaved between the β and γ phosphates. We therefore performed a series of experi-
**hGBP1-associated GTPase**

**Fig. 5.** hGBP1 fails to hydrolyze GDP. A control sample without hGBP1 (A) and with 0.125 μg/μl of purified hGBP1 (B) was incubated in the presence of 200 μM of unlabeled GDP. After 60 min at 37 °C, the reaction products were analyzed by HPLC. C and D, same as panels A and B, but the reaction was performed in the presence of GTP rather than GDP. The chromatogram was monitored at 252 nm. The identity of the nucleotide peaks and their retention times are indicated.

**Fig. 6.** hGBP1-catalyzed hydrolysis of GTP at lower temperature. GTPase assays were carried out at 37 °C (A) or 15 °C (B) in the presence of 0.125 μg/μl of hGBP1. A control reaction without hGBP1 was performed at 37 °C (C). The reaction buffer contained 200 μM unlabeled GTP. Analysis of the nucleotides after incubation for 1 h was performed by HPLC. The chromatogram was monitored at 252 nm. The identity of the nucleotide peaks and their retention times are indicated. The peak areas were integrated, and the relative concentrations of the various nucleotides (in percent) are given.

The C-terminal CaaX Motif of hGBP1 Serves as an Isoprenylation Signal—The C-terminal four amino acids of hGBP1, Cys-Thr-Ile-Ser, match the consensus sequence of CaaX motifs, which serve as signal sequences for isoprenylation (12). To test whether the CaaX motif of hGBP1 is functional, we determined whether mevalonic acid could be transferred to purified recombinant hGBP1 by incubation with reticulocyte lysate, which is known to contain an enzymatic activity that modifies proteins with CaaX motifs (28). After treatment with reticulocyte lysate and radiolabeled mevalonic acid, the histidine-tagged purified hGBP1 was recovered from the reaction mixture with Nickelate-agarose, eluted from the agarose beads with SDS, and...
analyzed by PAGE followed by Coomassie Blue staining (Fig. 9A) and autoradiography (Fig. 9B). hGBP1 treated in this manner became radioactive. This must have been due to bona fide isoprenylation of hGBP1 at the cysteine residue at position 589, because a mutant of hGBP1 (C598S) that has a serine at position 589 in place of cysteine remained unlabeled under these conditions (Fig. 9B). Similar experiments were performed with radiolabeled farnesyl pyrophosphate. Wild-type hGBP1 readily accepted this substrate, whereas the C598S mutant of hGBP1 did not (Fig. 9).

**DISCUSSION**

The IFN-induced human and mouse GBPs are known for their unusual guanine nucleotide-binding properties; they strongly bind to agarose-immobilized GTP, GDP, and GMP (20–22). Furthermore, unlike most other proteins with GTP binding or hydrolyzing activity, GBPs have a nontypical GTP binding motif; the third element of the tripartite motif is missing in the human and mouse GBPs (23). We now demonstrate that hGBP1 is a GTPase that converts GTP to GMP. Hydrolysis most likely occurs via two consecutive cleavages of single phosphate groups. This unusual biochemical property emphasizes the unique position of GBPs in the superfamily of GTPases.

hGBP1 was expressed as a histidine-tagged protein in *E. coli*, offering the possibility to purify this protein conveniently by Ni-chelate-agarose chromatography under nondenaturing conditions. Recombinant hGBP1 with a histidine tag at its N terminus and hGBP1 from IFN-treated human cells bound comparably well to agarose-immobilized guanine nucleotides, suggesting that the recombinant material exhibits normal biochemical activity. *E. coli*-derived hGBP1 thus seems to qualify as convenient starting material for a detailed analysis of its biochemical and structural properties. The hGBP1-mediated conversion of GTP to GMP occurred at a rather high rate of about six/min. hGBP1 performed multiple cycles of GTP hydrolysis in the absence of exchange factors and other accessory proteins; the *Kₐ* value for this reaction was about 470 μM. Since the intracellular GTP concentration was estimated to be about 100 μM (2), the *in vivo* activity of hGBP1 may be somehow lower than its theoretical maximum. Thus, in many respects, hGBP1 resembles some other high molecular weight GTPases, for instance, Mx proteins (29) and dynamin (30), which also feature high intrinsic GTPase activity and characteristically high *Kₐ* values.

**Fig. 7.** Pyrophosphate does not accumulate during hGBP1-catalyzed hydrolysis of GTP. GTPase assays were carried out at 37 °C for 5 and 15 min in the presence of 0.025 μg/ml of MonoQ-purified hGBP1 in a final volume of 20 μl containing 90 μM GTP; 12 nM [γ-32P]GTP, and various concentrations of pyrophosphate, namely 10 μM (lanes 1 and 5), 30 μM (lanes 2 and 6), 100 μM (lanes 3 and 7), or none (lanes 4 and 8). Samples were analyzed by paper chromatography to separate monophosphate and pyrophosphate. The relative positions of GTP and monophosphate and pyrophosphate markers are indicated.

**Fig. 8.** GTPγS inhibits hydrolysis of GTP but is no substrate of hGBP1. A, GTPase assays were carried out at 37 °C for 20 min in the presence of 0.125 μg/ml of hGBP1 in a final volume of 20 μl. The reactions contained 12 nM [α-32P]GTP and 100 μM GTP plus various amounts of unlabeled GTP or GTPγS. No competitor (lane 1), 2-fold excess of GTP or GTPγS (lanes 2 and 6), 5-fold excess of GTP or GTPγS (lanes 3 and 7), 10-fold excess of GTP or GTPγS (lanes 4 and 8), 15-fold excess of GTP or GTPγS (lanes 5 and 9). Other GTPase assays were carried out at 37 °C for 30 min in the absence (B) or presence (C) of 1.5 μg of hGBP1 in 30 μl of buffer that lacked GTP but contained 200 μM GTPγS. Product analysis was done by HPLC, and the chromatogram was monitored at 252 nm. The signal of GTPγS is indicated. The asterisk marks the signal of a contaminating nucleotide present in commercially available GTPγS. The expected position of GMP in panel C is marked on the chromatogram by an arrow.
The biochemical parameters of the hGBP1-mediated hydrolysis of GTP were quite unexpected in the light of the fact that hGBP1 bound very tightly to agarose-immobilized guanine nucleotides. The observed high $K_m$ value and the multi-cycle nature of the hydrolysis reaction rather suggested a weak association between hGBP1 and its substrates and products. In fact, filter binding assays indicated that hGBP1 has a low affinity for soluble GTP. Similarly, the binding studies with agarose-immobilized guanine nucleotides suggested that hGBP1 can bind GDP. However, experiments with nonimmobilized GDP showed that this nucleotide could neither serve as substrate of hGBP1 nor as competitor of the hGBP1-catalyzed GTP hydrolysis reaction. We have no satisfactory explanation for these discrepancies. It is possible that the commercially available GDP-agarose contained substantial amounts of GMP or GTP. Further, the immobilized guanine nucleotides, which are attached to the agarose beads at the ribose hydroxyls via a spacer, may have slightly altered overall structures that could explain the observed differences.

Hydrolysis of GTP to GMP by hGBP1 could be the result of two consecutive cleavages of single phosphate groups or, alternatively, a single cleavage of one pyrophosphate. Our data strongly favor the first possibility. First, we failed to detect pyrophosphate among the reaction products. Second, hGBP1 failed to hydrolyze GMP-PNP and GTPyS, GTP analogs with cleavage-resistant bonds between the $\beta$ and $\gamma$ phosphates. The failure of hGBP1 to hydrolyze these nucleotides was probably not due to poor binding, because they were efficient inhibitors of the hGBP1-catalyzed hydrolysis of GTP. Third, GDP was a reaction product which it should not be if hGBP1 indeed degraded GTP to GMP and pyrophosphate. The relative amounts of GDP product increased at low temperature, suggesting that it was released prematurely from the enzyme under sub-optimal conditions. This result suggests that cleavage of the second phosphate is the rate-limiting step.

The sequence Cys-Thr-Ile-Ser at the C terminus of hGBP1 matches the consensus sequence of CaaX motifs, which serve as signal sequences for isoprenylation (12). Our in vitro isoprenylation assays established that the CaaX motif of hGBP1 is functional. Radiolabeled mevalonic acid could be transferred to wild-type hGBP1 but not to a mutant form of hGBP1 with a Cys to Ser exchange. Farnesyl pyrophosphate could also be transferred to hGBP1, suggesting that this form of isoprenylation may occur naturally. hGBP1 thus joins the rapidly growing group of proteins that carry a farnesyl modification at the C terminus. Interestingly, this group includes p21ras (8–10) and heterotrimeric G proteins (11) as well as GTPases involved in the signal transduction process. Isoprenylation anchors these proteins to cell membranes, a process that seems to be necessary for proper in vivo activity (8, 9). Our in vitro isoprenylation studies suggest that hGBP1 of IFN-treated human cells may be farnesylated and that hGBP1 may be associated with membranes. Indirect immunofluorescence analysis of IFN-treated human cells and transfected mouse cells with antibodies specific for hGBP1 showed a punctate staining pattern, as would be expected if hGBP1 was associated with inner cell membranes.

Acknowledgment—We thank Annette Schwarz for expert technical assistance.

REFERENCES


2. M. Schwemmle and M. Bachmann, unpublished results.