Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 6303–6307, July 1991
Biochemistry

Identification of a human γ-glutamyl cleaving enzyme related to, but distinct from, γ-glutamyl transpeptidase

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Communicated by Irwin Fridovich, March 25, 1991 (received for review December 3, 1990)

ABSTRACT **We have cloned a 2.4-kilobase cDNA from a human placental cDNA library by using a γ-glutamyl transpeptidase [GGT; γ-glutamyltransferase, (5-glutamyl)-peptide:amino acid 5-glutamyltransferase, EC 2.3.2.2] probe. The deduced amino acid sequence of this cDNA, GGT-rel, exhibited an overall similarity of 39.5% with human GGT. Sequences that could represent a heavy and a light chain, analogous to GGT, as well as a putative transmembrane region were identified in GGT-rel. Transfectants overexpressing GGT-rel were tested for their ability to catalyze cleavage of the γ-glutamyl moiety from natural and synthetic substrates for GGT. Experiments with glutathione added to the medium suggested that GGT-rel could hydrolyze the γ-glutamyl moiety. More definitive evidence was obtained in experiments in which this protein converted leukotriene C_4 to leukotriene D_4. However, GGT-rel did not convert synthetic substrates that are commonly used to assay GGT. Our results indicate that GGT can no longer be considered the only enzyme capable of cleaving the γ-glutamyl linkage of leukotriene C_4 and, most likely, of other natural compounds.**

γ-Glutamyl transpeptidase [GGT; γ-glutamyltransferase, (5-glutamyl)-peptide:amino acid 5-glutamyltransferase, EC 2.3.2.2] is one of the major enzymes involved in the metabolism of glutathione (L-γ-glutamyl-L-cysteinylglycine; GSH). GSH participates in many important biological processes including biosynthesis of proteins, leukotrienes, and DNA as well as enzyme activation and cellular protection. GSH contains an unusual γ-glutamyl peptide bond. GGT is considered the only enzyme capable of cleaving this linkage in GSH and in other γ-glutamyl compounds; in addition, it can transfer the γ-glutamyl moiety to acceptor molecules in a transpeptidation reaction (1).

The enzyme consists of a heavy subunit (nonglycosylated, M_r = 41,650), which is membrane-associated, and a light subunit (nonglycosylated, M_r = 19,750), which contains the catalytic activity of the molecule (2–4). Both subunits are located on the external surface of cells; the enzyme is especially active in epithelial cells, which are involved in secretory and absorptive functions (5, 6).

The regulation of the expression of the GGT gene is likely to be complex and on many levels. Rat kidney and liver cDNAs appear to be almost identical in the nucleotide sequence of their coding regions but differ in their 5′ untranslated region. Therefore, they probably originated through alternative splicing from a single locus (7–9).

In humans, a family of at least four GGT genes exists on chromosome 22 (10, 11). A minimum of two of these appear to be transcribed, since a human kidney cDNA has been isolated that differs from the placental and liver cDNAs (12).

There is a tissue-specific distribution of GGT: the highest level is found in adult kidney. Adult hepatic GGT can be induced by glucocorticoids in rats (13), chronic alcoholism in humans (14), and carcinogens and drugs (15–17). GGT levels are also increased in the mammary gland during lactation and in rat uterus during the decidual response (18, 19).

In experiments designed to isolate a complete human GGT cDNA, we have cloned a cDNA that hybridized to, but was distinct from, GGT. In the current study, we have investigated the structure and function of this GGT-related (GGT-rel) gene.¶ Our results indicate that this gene has a significant structural similarity to GGT yet differs substantially from GGT in some, but not all, biological aspects.

MATERIALS AND METHODS

Isolation of cDNA Clones and Sequencing. A partial 1.8-kilobase (kb) cDNA, hpl 14-1, was isolated from a human placental cDNA library (Clontech) by using a previously described (11) GGT cDNA probe. A full-length cDNA of 2.4 kb, 9hpl3A-2, was isolated from the same library by using an hpl 14-1 probe. The nucleotide sequence was obtained for both DNA strands as described (20).

Expression Constructs. The complete insert of 9hpl3A-2 was subcloned into a modified pCDX (21) vector. DNAs were cotransfected with pSV2-neo essentially as described (22).

Northern and Southern Blot Analyses. Isolation of RNA, DNA, and probes as well as hybridization were as described (23, 24). A 0.27-kb *Eco*RI–*Hin*cII fragment from hpl 14-1 encompassing bases 395–585 (see Fig. 1) was used as the probe in Fig. 4; other GGT-rel probes included a 0.37-kb *Eco*RI–*Sst* I fragment from hlp 14-1 cDNA encompassing bases 395–770 for Southern blots (Fig. 3*A*) and the entire hpl 14-1 *Eco*RI insert for Northern blots (Fig. 3*B*). GGT probes were the entire clone 4A-2 *Eco*RI insert and a 0.48-kb *Eco*RI–*Pvu* II fragment encompassing bases 670–1155 (11).

Antisera and Western Blot Analysis. A 0.6-kb *Sst* I–*Taq* I restriction enzyme fragment from the putative heavy chain (residues 143–340; see Fig. 1) was ligated in-frame into the trpE fusion protein vector pATH 11. Antisera were obtained as described (24). Western blot analysis was performed on cellular extracts with 20 μg of total protein, primary antibody was used at a dilution of 1:1000, and immunoreactive material was visualized by using goat anti-rabbit IgG conjugated to alkaline phosphatase (Bio-Rad).

Leukotriene C_4 (LTC_4) Conversion. Equal numbers of murine NIH 3T3 control cells or NIH 3T3 cells transfected with the GGT construct (3T3/GGT cells) or with the GGT-rel construct (3T3/GGT-rel cells) were incubated in serum-free and antibiotic-free Dulbecco's modified Eagle's medium supplemented with 6 μM LTC_4 for 2 hr at 37°C. One-tenth-milliliter aliquots of medium were filtered and diluted 1:1 in ethanol and analyzed by HPLC. Conversion of leukotrienes

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Abbreviations: GGT, γ-glutamyl transpeptidase; GSH, glutathione; GGT-rel, GGT related; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M64099).

was also analyzed by using plasma membrane preparations and bovine kidney GGT (Sigma). Ten micrograms of the membrane preparations and 2 μg of purified GGT were incubated with 5 μM LTC_4 in water for 10 min, 20 min, 1 hr, and 2 hr at 37°C. Samples were separated on a C_{18} reverse-phase column (Waters or Millipore) as described (25).

Glutathione Degradation. Ten micrograms of crude membrane preparations of 3T3 control and 3T3/GGT-rel cells was incubated with 0.2 mM GSH with or without 0.2 mM glycylglycine for 1 and 6 hr at 37°C. The samples were precipitated with 10% perchloric acid and 2 mM EDTA containing 0.1 mM γ-glutamylglutamic acid (as an internal control). After centrifugation, the supernatants were derivatized with iodoacetic acid, 2 M KOH, 2.4 M $KHCO_3$, and 1% fluorodinitrobenzene in ethanol and analyzed by HPLC.

RESULTS

Isolation and Analysis of GGT-rel cDNA. By using a human GGT cDNA probe, a 1.8-kb cDNA was isolated from a human placental cDNA library using relatively stringent [0.3× standard saline citrate (SSC), 65°C] posthybridization washing conditions. Although this suggested that the newly isolated cDNA should have substantial homology to the GGT cDNA, restriction enzyme analysis indicated that it was completely different. A second cDNA of 2.4 kb was isolated by using the 1.8-kb cDNA as a probe.

Sequence analysis of these clones revealed a long open reading frame commencing at nucleotide 341 and continuing up to a stop codon at nucleotide 2100 (Fig. 1). The nucleotides surrounding the designated initiator methionine codon, GCCATGG, match the consensus sequence for an initiation codon (26). The long open reading frame has the capacity to encode a protein of 586 amino acids. There is a relatively large 5′ untranslated region of 340 nucleotides. This region has a special sequence organization: numerous direct repeats consisting of 8–12 nucleotides are found, which could play a role in transcriptional or translational regulation (for example, see Fig. 1, repeats 1–3). We compared its deduced amino

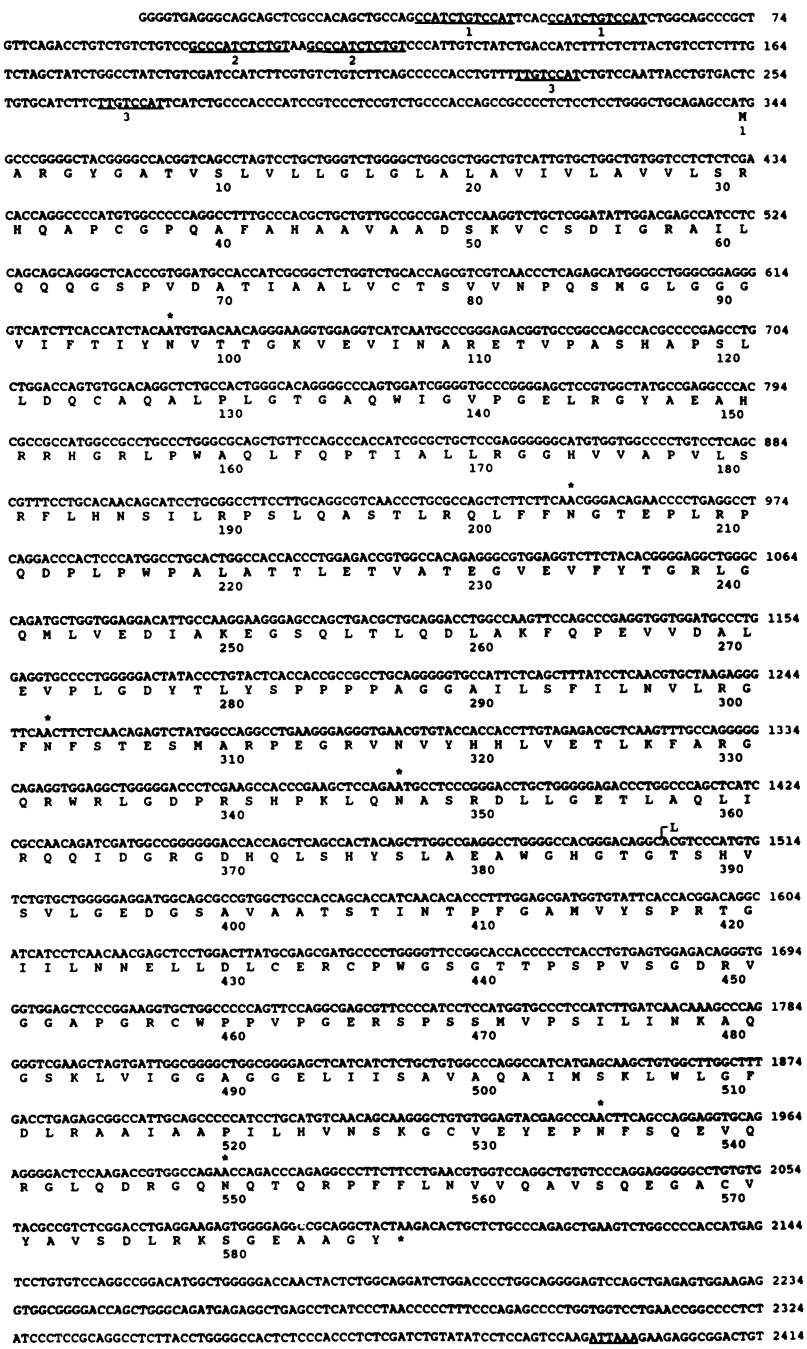

FIG. 1. Nucleotide and deduced amino acid sequence of GGT-rel. Direct repeats in the 5′ untranslated region are underlined and numbered 1–3. Asterisks denote possible N-linked glycosylation sites. An L above the sequence at amino acid position 388 points to the amino terminus of the putative light chain. The underlined sequence indicates the polyadenylylation signal.

acid sequence with that of human GGT (11). Of 586 amino acid residues, 231 (39.5%) were identical to those of GGT (Fig. 2). The GGT gene encodes its heavy and light chains on a single transcript; the amino-terminal amino acid of the light chain is at position 381 (Fig. 2). If one assumes that the related gene has a similar organization (also see below), the threonine at residue 388 would be the amino terminus of its light chain. The identity between the heavy and light chains of these gene products (154 of 387 residues, or 39.6%, and 77 of 198 residues, or 39.8%, respectively) would be comparable to their overall homology, indicating that this is not restricted to certain areas of the molecule (e.g., its putative active center). The calculated molecular weight of the entire protein would be 62,300; the putative heavy and light chains of the GGT-rel protein would be 41,500 and 20,800, respectively; this is comparable to the corresponding molecular weights of the GGT subunits (41,650 and 19,750). The putative heavy chain contains four and the light chain contains two possible N-linked glycosylation sites (see Fig. 1). If fully glycosylated, the molecular weight of the putative heavy chain would be ≈53,500 and that of the light chain would be ≈26,800.

A hydropathy analysis exhibited four regions with a hydropathy index greater than 1.6 located in positions very similar to those in GGT (data not shown). Transmembrane regions were predicted by using the ALOM program (11). Only one of these four hydrophobic domains (amino acids 12–28; maximum extension 4–34) was found to have a high probability (4000:1) of being transmembrane. Previously, a similar region of GGT was identified as being transmembrane (11).

Expression of GGT-rel. Since the analysis of the primary structure of GGT-rel indicated that it was homologous to GGT, the questions of similarity in expression and activity were also addressed.

On a genomic level, the GGT gene belongs to a family of at least four members (10, 11). A 0.48-kb *Eco*RI–*Pvu* II probe prepared from the 5′ coding region of a GGT cDNA hybridized to at least five fragments in *Bam*HI-digested human genomic DNA (Fig. 3*A*, lane 3). In contrast, a 0.37-kb *Eco*RI–*Sst* I probe from a similar position in the GGT-rel cDNA detected three *Bam*HI fragments of 2.1–1.9 kb and two fragments of 6.4 and 6 kb (Fig. 3*A*, lane 2). Since comparable probes from GGT and GGT-rel hybridized to different fragments, it is clear that, using relatively stringent posthybridization conditions, the GGT-rel probe does not detect members of the GGT gene family. The multiple bands detected with the GGT-rel probe with different enzymes such as *Bam*HI and *Eco*RI (Fig. 3*A*, lanes 2 and 5) suggest that GGT-rel is a member of a gene family. GGT-rel is conserved in evolution, since the probe detected a limited amount of *Eco*RI fragments in mouse DNA under nonstringent posthybridization washing conditions (Fig. 3*A*, lane 6) and a single fragment after stringent washing (Fig. 3*A*, lane 7). In spite of this conservation and, although a human GGT probe detected relatively high levels of the 2.4-kb GGT mRNA in adult mouse kidney and fetal liver, no expression of the GGT-rel gene was detected by using low-stringency posthybridization washings in any mouse tissues tested, including adult brain, eye, heart, lung, thyroid, bladder, intestine, kidney, liver, pancreas, spleen, bone marrow, salivary glands, and 12-day-old embryonic kidney, liver, placenta, and total embryo (data not shown).

Expression was also examined in a panel of RNAs isolated from various human cell lines. With a GGT probe, mRNA was readily detected in four of five cell lines tested (Fig. 3*B Left*). The GGT-rel probe showed no hybridization to RNA isolated from lymphoblast cell lines (IM-9 and GM 7441), pulmonary fibroblasts, the kidney carcinoma cell line A498 (Fig. 3*B Right*), a lung carcinoma cell line (ChaGo), or a glioblastoma (A172). However, an mRNA species slightly larger than the GGT mRNA (≈2.7 kb) was visible upon prolonged exposure in the cell lines K562 (erythroleukemia) and HepG2 (hepatoblastoma) (Fig. 3*B Right*, lanes 2 and 5). Thus, most tissues and cell lines examined lacked abundant expression of the GGT-rel gene.

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1   MARGYGATVSLVLLGLGLALAVIVLAVVLSRHQAPCGPQAFAHAAVAADSKVCSDIGRAI
          : : ::   : :    :   :          :::::: : :: :::
1'     MKKKLVVLGLLAVVLVLVIVGLCLWLPSASKEPDNHVYTRAAVAADAKQCSKIGRDA

61  LQQQGSPVDATIAALVCTSVVNPQSMGLGGGVIFTIYNVTTGKVEVINARETVPASHAPS
    :   :: ::: :::: :    :  ::: :::   :::: :: : ::::::::  :
58' LRDGGSAVDAAIAALLCVGLMNAHSMGIGGGLFLTIYNSTTRKAEVINAREVAPRLAFAT

121 LLDQCAQALPLGTGAQWIGVPGELRGYAEAHRRHGRLPWAQLFQPTIALLRGGHVVAPVL
        :    :     :::: ::::  :: :::::::: :::: : : : :   :
118'MFNSSEQ---SQKGGLSVAVPGEIRGYELAHQRHGRLPWARLFQPSIQLARQGFPVGKGL

181 SRFLHNSILRPSLQASTLRQLFFNGTEPLRPQDPLPWPALATTLETVATEGVEVFYTGRL
     : :     :   :   :      ::    : : :: : :: :: ::   :: : :
175'AAALENKRTVIEQQ-PVLCEVFCRDRKVLREGERLTLPQLADTYETLAIEGAQAFYNGSL

241 GQMLVEDIAKEGSQLTLQDLAKFQPEVVD-ALEVPLGDYTLYSPPPPAGGAILSFILNVL
      : ::   :   :  ::     :    :  :::  :: :  :  :  :  ::: :
234'TAQIVKDIQAAGGIVTAEDLNNYRAELIEHPLNISLGDAVLYMPSAPLSGPVLALILNIL

300 RGFNFSTESMARPEGRVNVYHHLVETLKFARGQRWRLGDPRSHPKLQNASRDLLGETLAQ
     : ::: ::   ::     ::  ::   ::   :  ::::                ::
294'KGYNFSRESVESPEQKGLTYHRIVEAFRFAYAKRTLLGDPKFVDVTEVVRNMTSEFFAAQ
                          *
360 LIRQQIDGRGDHQLSHYSLAEAWGHGTGTSHVSVLGEDGSAVAATSTINTPFGAMVYSPR
    : : ::    :  : :    :: : ::  ::::: ::::::  ::  : ::
354'L-RAQISDDTTHPISYYKPEFYTPDDGGTAHLSVVAEDGSAVSATSTINLYFGSKVRSPV
                          *
420 TGIILNNELLDLCERCPWGSGTTPSPVSGDRVGGAPGRCWPPVPGERSPSSMVPSILINK
     ::  :::  :              ::     :  :       ::    ::: : :
413'SGILFNNEMDD-----------FSSPSITNEFGVPPSPANFIQPGKQPLSSMCPTIMVGQ

480 AQGSKLVIGGAGGELIISAVAQAIMSKLWLGFDLRAAIAAPILHVNSKGCVE-YEPNFSQ
        : : :::  :  : : ::   :: : :   :  : ::      :  : :  :
462'DGQVRMVVGAAGGTQITTATALAIIYNLWFGYDVKRAVEEPRLHNQLLPNVTTVERNIDQ

539 EVQRGLQDRGQNQTQRPFFLNVVQAVSQEGACVYAVSDLRKSGEAAGY
    :  :   :    :  ::::          : :: :: :: :::
522'AVTAALETRHHHTQIASTFIAVVQAIVRTAGGWAAASDSRKGGEPAGY
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FIG. 2. Comparison of deduced amino acid sequences of GGT and GGT-rel. The upper line contains the GGT-rel sequence, the lower line contains the sequence of human GGT (11). The asterisks at position 381 in GGT and position 388 in GGT-rel mark the amino termini of their light-chain sequences.

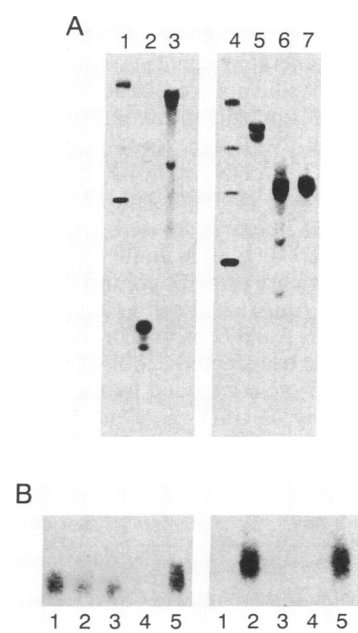

FIG. 3. Southern and Northern blot analyses of GGT-rel. (*A*) DNAs were digested with *Bam*HI (lanes 2 and 3) or *Eco*RI (lanes 5–7) and hybridized to a 0.37-kb *Eco*RI–*Sst* I GGT-rel probe (lanes 2, 5–7) or a 0.48-kb *Eco*RI–*Pvu* II GGT probe (lane 3). Human DNAs are in lanes 2, 3, and 5; mouse DNAs are in lanes 6 and 7. Posthybridization washings were at 65°C in 0.3× SSC (lane 2), 2.5× SSC (lanes 3 and 6), or 0.01× SSC (lanes 5 and 7). Exposure times for lanes 6 and 7 were 6 and 48 hr, respectively. *Hin*dIII-digested λ molecular weight markers are shown in lanes 1 and 4. (*B*) RNAs from the human cell lines A498 (lanes 1), K562 (lanes 2), ChaGo (lanes 3), A172 (lanes 4), and HepG2 (lanes 5) were first hybridized to a GGT probe (*Left*) and subsequently to a GGT-rel probe (*Right*). Exposure times were 14 hr (*Left*) and 7 days (*Right*).

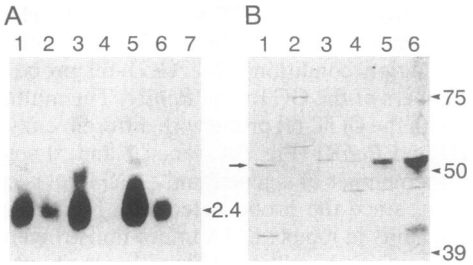

FIG. 4. GGT-rel transfectants. (*A*) Northern blot analysis. RNAs include those from the pool of transfectants (lane 1), individual colonies (lanes 2–6), and control NIH 3T3 cells (lane 7). The position of the 2.4-kb GGT-rel mRNA is indicated. (*B*) Western blot analysis. Cells include those from HepG2 induced by dexamethasone (lane 1), uninduced HepG2 cells (lane 2), 3T3/GGT-transfected cells (lane 3), untransfected, control NIH 3T3 cells (lane 4), 3T3/GGT-rel transfectant 2 (lane 5), and 3T3/GGT-rel transfectant 4 (lane 6). The positions of molecular weight standards ($\times 10^{-3}$) are indicated to the right; an arrow to the left of *B* points to the prominent M_r 55,000 proteins possibly representing the glycosylated GGT-rel heavy chain. Note that approximately three times more protein was loaded in lane 2 than in lane 1.

To study the biological function of GGT-rel, however, it was important to have a cell line available that produces substantial amounts of protein. To this end, the complete GGT-rel cDNA was inserted into a eukaryotic expression vector. This construct, driven by the simian virus 40 promoter/enhancer, produced substantial amounts of mRNA in the pool of transfectants (Fig. 4*A*, lane 1) and in several individual colonies after G418 selection (Fig. 4*A*, lanes 2–6); there was no detectable signal in untransfected NIH 3T3 cells (Fig. 4*A*, lane 7).

To examine the GGT-rel protein, a segment of the putative heavy chain was used to produce a bacterial fusion protein, and antisera were generated against it. The antiserum appears to be specific, since it did not cross-react with GGT ≈200-fold overproduced (3T3 control, 4.8 ± 2.9 units/g of protein; 3T3/GGT, 826.6 ± 292.0 units/g of protein) in a cell line transfected with a GGT cDNA (Fig. 4*B*, lane 3). The GGT-rel antisera did detect two proteins with M_r values of ≈55,000 and 42,000 in HepG2 cells incubated with 1 μM dexamethasone (Fig. 4*B*, lane 1) but not in uninduced HepG2 cells (Fig. 4*B*, lane 2). In comparison with control 3T3 cells (Fig. 4*B*, lane 4), two of the transfected cell lines (transfectants 2 and 4; see Fig. 4*B*, lanes 5 and 6) clearly produced proteins. This established that the transfected cell lines produced substantial amounts of a protein encoded by the GGT-rel cDNA.

Biological Activity of GGT-rel. One of the hallmarks of GGT activity is the ability to cleave and transfer the γ-glutamyl moiety of GSH and other γ-glutamyl compounds to acceptors (1). Therefore, we examined the activity of GGT-rel toward GSH. Identical numbers of transfected and control cells were incubated with 0.2 mM GSH added to the medium; samples of the medium (27) were assayed for GSH up to 24 hr after addition. In control NIH 3T3 cells, degradation of GSH was slow and did not exceed 15% during a 24-hr period; in contrast, the GGT-rel-transfected cell line rapidly degraded GSH (Fig. 5). In addition, filtered medium, in which 3T3/GGT-rel cells had grown for a period of 48 hr, was tested for GSH degradation activity. This medium contained negligible activity, indicating that, as GGT, GGT-rel is an extracellular membrane-bound protein.

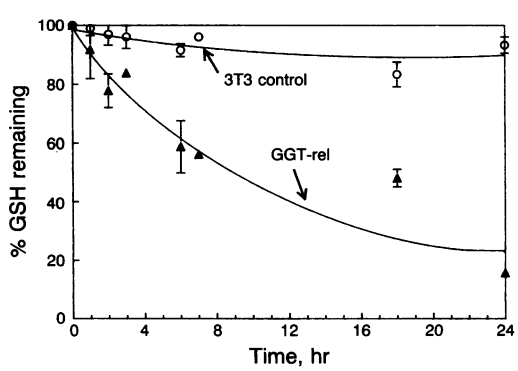

FIG. 5. Degradation of extracellular GSH by 3T3/GGT-rel and 3T3 control cells.

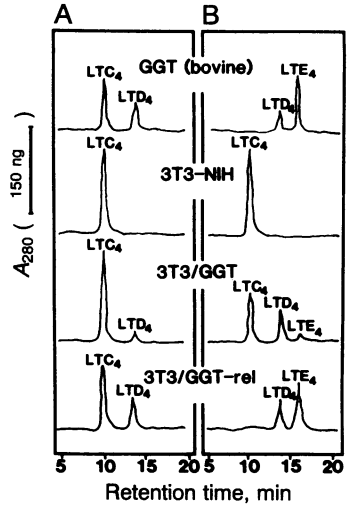

FIG. 6. Conversion of LTC_4 by GGT and GGT-rel. Partially purified preparations were made from control NIH 3T3, 3T3/GGT-transfected, and 3T3/GGT-rel-transfected cells. Commercially available GGT was used as a control. LTC_4 conversion after a 10-min (*A*) or 60-min (*B*) incubation is shown. The scale on the ordinate indicates an absorbance obtained with a known amount of LTC_4.

In addition to GSH, LTC_4 is a natural substrate for GGT (28). Unlike GSH, which potentially could be degraded by processes other than cleavage of the γ-glutamyl bond, LTC_4 can only be converted to leukotriene D_4 (LTD_4) by GGT. The product of this action of GGT, LTD_4, lacks the γ-glutamyl residue and is further metabolized by a dipeptidase to give leukotriene E_4 (LTE_4). However, LTE_4 can only be generated from LTD_4 and not from LTC_4. All three forms of leukotrienes are the components of the slow-reacting substance of anaphylaxis (29) and are involved in immediate hypersensitivity and inflammatory reactions (30). To investigate the possible activity of the GGT-rel protein in this pathway and to confirm our hypothesis that GGT-rel is capable of cleaving the γ-glutamyl bond, we analyzed the conversion of extracellularly added LTC_4 by 3T3/GGT-rel transfectants *in vivo*. LTC_4 was not converted by control NIH 3T3 cells; however, after an incubation of 2 hr with transfected cells (3T3/GGT-rel), a substantial amount of LTC_4 was converted into a product with the same retention time and UV spectrum as authentic LTD_4 (data not shown). The product was also indistinguishable from that produced by the reaction of LTC_4 with 3T3/GGT cells. The same numbers of cells were used for this experiment, indicating that both GGT- and GGT-rel-transfected cell lines did exhibit similar rates of LTC_4 to LTD_4 conversion during 2 hr of incubation.

We repeated this experiment with partially purified membrane preparations (31) of control and transfected cells. As a standard enzyme we used commercially available bovine kidney GGT. As shown in Fig. 6, the conversion of LTC_4 followed the pattern observed in the experiment with intact cells; LTD_4 was further degraded to LTE_4 by dipeptidase, which is associated with the cell membranes. The presence of the LTE_4 peak in the sample containing only purified bovine

GGT is most likely explained by the contamination of this commercial preparation with dipeptidase as was suggested by others (32). With intact GGT-rel-transfected cells, LTC_4 was converted only to LTD_4. When the cells were disrupted, LTE_4 was also produced. Although unlikely, we cannot exclude that GGT-rel could catalyze both steps.

GGT is commonly assayed using L-γ-glutamyl-*p*-nitroanilide and glycylglycine (33); the assay requires that the enzyme cleaves off the L-γ-glutamyl moiety and releases free *p*-nitroaniline. The enzyme produced by the GGT-transfected cell lines was extremely active in this assay; however, cells transfected with GGT-rel exhibited absolutely no activity (data not shown). Similarly, we found that γ-glutamyl-4-methoxy-2-naphthylamide cannot be cleaved by GGT-rel (data not shown). In conclusion, GGT-rel has the physiologically important property of catalyzing γ-glutamyl cleavage; however, its substrate specificity is clearly different from GGT.

Acivicin is a potent irreversible inhibitor of GGT. We investigated the effect of increasing concentrations of acivicin on GSH degradation by membrane extracts isolated from 3T3 control, 3T3/GGT, and 3T3/GGT-rel cells (data not shown). There was a marked inhibitory effect of acivicin on the ability of GGT-rel to degrade GSH, although this might have been slightly weaker than in the case of GGT.

DISCUSSION

We have identified a gene related to GGT. On an amino acid level, it is substantially homologous (40%) to the GGT protein. Our experiments on the degradation of exogenous GSH and conversion of LTC_4 to LTD_4 using intact cells strongly suggest that GGT-rel is likely to be an extracellular, membrane-associated enzyme.

We considered several possible, alternative functions for the GGT-rel protein such as one of the other enzymes of the GSH metabolism or a hitherto undiscovered enzyme. Although we cannot completely exclude the first alternative, GGT-rel has no homology with any of the enzymes cloned to date. The isolation of a cDNA supports several possibilities: (*i*) it is expressed at extremely low levels, (*ii*) its expression is induced by some unknown agent, or (*iii*) it is expressed in a very small population of specialized cells. The first possibility is substantiated by the detection of a low level of expression of GGT-rel in HepG2, a liver-derived cell line, and in the erythroleukemia cell line K562.

An interesting conclusion can be drawn from our experiments using synthetic substrates commonly used to detect GGT: GGT-rel apparently cannot utilize L-γ-glutamyl-3-carboxy-4-nitroanilide or γ-glutamyl-4-methoxy-2-naphthylamide. Indeed, even in our GGT-rel overexpressing cell lines, no increase in activity for these synthetic substrates was found, demonstrating that the ability to cleave the γ-glutamyl group, of GSH for example, is inherent to GGT-rel and is not mediated through a pathway that induces the normal GGT activity. Moreover, it follows that GGT-rel activity is not measured in assays commonly used for GGT.

The HPLC analyses of leukotriene conversion confirm the data obtained from the GSH degradation experiments; similar to GGT, GGT-rel is able to cleave the γ-glutamyl bond, since this represents the only difference between LTC_4 and LTD_4. Thus, GGT and GGT-rel have different but overlapping substrate specificities. GGT-rel is inhibited by acivicin, a known inhibitor of GGT. Recently, threonine-523 has been identified in rat kidney GGT as a specific acivicin-binding residue (34). Interestingly, there is no threonine in a homologous position in GGT-rel. However, the sequences of GGT and GGT-rel differ substantially; thus, any other threonine or even another amino acid containing a hydroxyl group may be the target of acivicin in the GGT-rel molecule.

Although GGT is apparently much more abundant than GGT-rel, this does not exclude an important physiological role for GGT-rel. Presently, we favor the hypothesis that GGT-rel may be an enzyme active in very specialized cell types or during certain stages of development, such as during pregnancy. GGT-rel was somewhat inducible by dexamethasone in the cell line HepG2, and the cDNAs were isolated from human placenta. While more experiments are necessary to elucidate the exact role of this enzyme in cellular processes such as the γ-glutamyl cycle, one important conclusion can now be drawn from our experiments: GGT itself can no longer be considered the only enzyme capable of cleaving the γ-glutamyl linkage of LTC_4 and probably GSH.

We thank Dr. T. Robison for help with the HPLC system and analysis of leukotrienes and T. Saluna and M. Brizuela for technical assistance. This work was supported in part by National Institutes of Health Grants HL 37556 to H.J.F. and CA 47456 to J.G.

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