Catalytic Triad of Monoglyceride Lipase

Monoglyceride lipase catalyzes the last step in the hydrolysis of stored triglycerides in the adipocyte and presumably also complements the action of lipoprotein lipase in degrading triglycerides from chylomicrons and very low density lipoproteins. Monoglyceride lipase was cloned from a mouse adipocyte cDNA library. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33,218. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33,218. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33,218. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33,218. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33,218. The predicted amino acid sequence consisted of 302 amino acids, corresponding to a molecular weight of 33,218.

The sequential hydrolysis of stored triglycerides in adipose tissue is the result of a combined action of two lipases, hormone-sensitive lipase and monoglyceride lipase (MGL; EC 3.1.1.23). Hormone-sensitive lipase catalyzes the first and rate-limiting step, the hydrolysis of triglycerides, and also the subsequent hydrolysis of di- and monoglycerides (1). Hormone-sensitive lipase has a marked, although not absolute, preference for the primary ester bond of glyceride substrates. It has been shown that MGL is required to obtain a complete degradation of monoglycerides to fatty acids and glycerol, i.e. in the absence of MGL there is an accumulation of monoglycerides (mainly 2-monoglycerides) (2). The main physiological role for MGL is probably to assure complete hydrolysis of monoglycerides formed during the lipolysis of stored triglycerides of the adipocyte. Another role for the enzyme could be to catalyze the hydrolysis of 2-monoglycerides formed as a result of lipoprotein lipase-catalyzed hydrolysis of triglycerides from chylomicrons and very low density lipoproteins. Lipoprotein lipase has monoglyceride-hydrolyzing activity, with an absolute preference for the primary ester bond (3). This lipase could therefore catalyze the hydrolysis of 1(3)-monoglycerides, which are formed through isomerization from 2-monoglycerides. However, since the rate of isomerization at pH 7.4 is low, it is more likely that a substantial fraction of the 2-monoglycerides, formed through the action of lipoprotein lipase, is transported into the adipocyte and hydrolyzed by MGL (4). It should be pointed out that besides these two enzymes, there is no evidence for any other monoglyceride-hydrolyzing activity of adipose tissue.

MGL has been extensively purified from rat adipose tissue in our laboratory (5). The limited amounts of purified enzyme obtained have been used to study some of its enzymological and biochemical properties. These studies have shown that MGL is a 32.9-kDa protein. Nonionic detergent is strictly required to purify MGL from adipose tissue and to keep it stable in aqueous solution in the purified state, indicating that the enzyme has an amphiphilic character, as has also been shown for hormone-sensitive lipase (6). With regard to enzymological properties (5), MGL appears to be a specific monoacylglycerol hydrolase, hydrolyzing the 1(3)- and 2-ester bonds at equal rates. Inhibition by micromolar concentrations of disopropylfluorophosphate indicates the presence of a reactive serine at the active site, as is the case for many lipases and esterases. Its activity is also extremely sensitive to inhibition by p-chloromercuribenzoic acid and mercury chloride, suggesting the presence of one or several essential sulfhydryl groups.

To date, many lipases and esterases have been cDNA cloned. Several of the known sequences show identity to each other, indicating evolutionary relationships and allowing the description of different families of lipases/esterases. However, no cDNA containing the entire coding sequence for a specific monoglyceride-hydrolyzing enzyme from any species or tissue has yet been described.

With regard to the three-dimensional structure of lipases, much has been learned in the recent years through x-ray crystallographic studies of several microbial lipases and one mammalian lipase, pancreatic lipase. The structures of these lipases show that they share a similar three-dimensional fold, called the α/β-hydrolase fold (7). This fold consists of a central β-sheet, surrounded by a variable number of α-helices. In turns between β-strands and α-helices, the catalytic triad, composed of a serine, a carboxylic acid, and a histidine, is found. The serine of the catalytic triad is, with a few exceptions, found...
within a GXSXG consensus sequence. The order of the residues of the catalytic triad in the primary sequence is serine followed by the carboxylic acid and the histidine in all lipases where the primary sequence as well as the residues of the catalytic triad are known (8, 9).

Despite the fair number of known lipase structures, including some structures solved of lipases complexed to substrates and substrate analogues, and several molecular models of lipases, little has been learned regarding the structural basis for substrate specificity of lipases. As part of a long term goal to understand the relationship between structure and substrate specificity, we decided to cDNA clone the MGL of adipose tissue. As stated above, this enzyme has been shown to be a specific monoacylglycerol hydrolase with no preference for either isomer. MGL should therefore be a good model enzyme for this type of study.

We describe in this report the complete amino acid sequence of mouse adipose tissue MGL. The sequence, which represents the first known MGL sequence, reveals that this enzyme is related to a number of microbial proteins that include esterases, lysophospholipases, and haloperoxidases. By means of sequence alignments and site-directed mutagenesis experiments, we have identified the residues of the catalytic triad and localized the secondary structure elements that constitute the α/β-hydrolase fold of this lipase. Finally, we show that the cloned MGL is not specific for adipose tissue, but seems to be widely expressed among tissues.

EXPERIMENTAL PROCEDURES

Purification of Rat MGL—To obtain large quantities of pure enzyme protein, a modification of our previously described purification scheme was employed (5). In brief, fractions containing active MGL from the QAE-Sephadex chromatography step of multiple purifications of hormone-sensitive lipase from epididymal rat fat pads (from a total of 5000 rats) were pooled (1). This pool (6 liters), contained in a buffer with the nondialyzable, nonionic detergent C12E7 and glycerol, was concentrated 8-fold by ultrafiltration (Filtrone Minisette, Omega 30K filter). The concentrate was divided into two portions, each of which was subjected to isoelectric focusing with an Ampholine gradient of pH 3.5–10, using a 440-ml column (LKB 8100 Ampholine column, Pharmacia Biotech). Peak activity fractions from both purifications were pooled and subjected to a second isoelectric focusing step. The peak containing the active MGL was pooled, dialyzed, and concentrated to 35 ml by ultrafiltration (PM-30 Diaflo membrane). This material was loaded into the bottom of a Sephadex G-200 superfine column (18 cm, Pharmacia Biotech) and eluted with reverse flow (6 ml/h), to prevent the sample with high density and viscosity from passing through the column by gravity. MGL activity was eluted as a single peak, and the corresponding fractions were pooled and ultrafiltrated to 55 ml. Finally, this material was included in an isoelectric focusing column (LKB 8110, 110 ml) with an Ampholine gradient of pH 5–8. The purity of the 32.9-kDa MGL protein in the peak fractions was checked by SDS-PAGE stained with 0.05% Coomassie Blue in 50% methanol, 7% acetic acid for 5 min and then destained in the same solution without dye. The band containing MGL was excised, transferred to an Eppendorf tube, and subjected to in-gel digestion according to Laemmli (10) and silver staining. MGL activity was measured using mono-[^H]oleate (MO) as substrate (11).

Internal Peptide Sequence Analysis—Forty μl of fraction 55 (Fig. 1), containing 100 pmol of MGL protein, were reduced and alkylated with dithiothreitol and iodoacetamide, respectively. The sample was subjected to SDS-PAGE (10), stained with 0.05% Coomassie Blue in 50% methanol, 7% acetic acid for 5 min and then destained in the same solution without dye. The band containing MGL was excised, transferred to an Eppendorf tube, and subjected to in-gel digestion according to Hellman et al. (12). In brief, the gel piece was washed with 0.2 M ammonium bicarbonate and 50% acetonitrile and then completely dried. During rehydration, 0.5 μg of modified trypsin (sequence grade; Promega) was added, and 0.2 M ammonium bicarbonate was given in small aliquots until the gel piece was immersed. After overnight incubation, the supernatant was saved and combined with extractions from the gel piece. Generated peptides were isolated by reversed phase HPLC on a μRPC C2/C18 SC 2.1/10 column in a SMART System (Pharmacia Biotech). Peptides were sequenced on a model 470A sequencer (Applied Biosystems), following the manufacturer’s instructions.

2C12E7 is a heterogeneous preparation of an alkyl polyoxyethylene ether detergent with the indicated average composition, where C represents alkyl carbons and E represents oxyethylene units (Berol 058, Berol kemi AB, Stenungsund, Sweden).
Monoglyceride lipase (MGL) is a lipolytic enzyme that hydrolyzes monoacylglycerols to free fatty acids and glycerol. It is involved in the digestion of triglycerides in adipose tissue and can be found in various organs, including the liver, pancreas, and small intestine.

MGL is a member of the lipase family and contains a serine catalytic triad (Ser, Asp, and His) that is essential for its catalytic activity. The active site of MGL is located in a cleft formed by the enzyme's secondary structure, which is stabilized by hydrogen bonds and hydrophobic interactions.

The catalytic activity of MGL is dependent on the pH and temperature of the environment. It is most active at pH 7.0-8.0 and at temperatures between 30°C and 40°C.

The cloning and expression of MGL have been studied extensively. Researchers have used various techniques, such as reverse transcriptase-polymerase chain reaction (RT-PCR) and complementary DNA (cDNA) cloning, to identify and isolate the MGL gene from different tissues.

Northern blot analysis has been used to quantitate MGL mRNA levels in various tissues. The results showed that MGL mRNA is expressed at high levels in the liver, pancreas, and small intestine, and at lower levels in other tissues.

expression of the cloned MGL gene in COS cells has been demonstrated. The recombinant MGL protein expressed in COS cells had similar enzymatic properties to the native enzyme, including substrate specificity and pH dependence.

Mutations in the MGL gene have been associated with certain diseases, such as lipemia retinæ, a hereditary disorder characterized by retinal deposits of lipids.

In conclusion, the cloning and expression of MGL have provided valuable insights into the structure and function of this enzyme. Further studies are needed to fully understand the role of MGL in lipid metabolism and its potential use in the treatment of lipid-related disorders.

FIG. 2. HPLC chromatograph of peptides obtained after tryptic digestion of rat MGL. AU, absorbance units.

FIG. 3. The nucleotide sequence and deduced amino acid sequence of mouse MGL. The tryptic peptide sequences obtained from the purified rat enzyme are underlined. The serine, the two aspartic acids, and the four histidines, which were mutated to probe the residues of the catalytic triad, are marked with circles, where the closed circles indicate the residues predicted to form the catalytic triad. The HG dipeptide is boxed.

Northern blot (2 μg poly(A)+ RNA/lane, CLONTECH) were hybridized with a probe corresponding to the coding part of the MGL cDNA, internally radiolabeled with [32P]dCTP, using the ExpressHyb System (CLONTECH). Membranes were analyzed by digital imaging using a Fujix Bas 2000 (Fuj1).

RESULTS

Cloning of Mouse MGL cDNA—Purified MGL was obtained in large quantities by upscaling and slightly modifying the original procedure described by us (5). The original procedure included detergent solubilization of a pH 5.2 precipitate from a fat-free 110,000 g supernatant of a rat adipose tissue homogenate, ion-exchange chromatography, gel exclusion chromatography, and finally two sequential isoelectric focusing steps, using a pH 6–8 gradient. For the present purification, our starting material consisted of pooled fractions containing MGL activity from the QAE chromatography step (corresponding to the ion exchange chromatography step mentioned above) of several purifications of hormone-sensitive lipase (see Fig. 1 in Ref. 1), which had been saved and stored at −20 °C. Concentration of this material (6 liters) in a buffer with a nondialyzable, nonionic detergent and glycerol to a volume of 35 ml was achieved by ultrafiltration and successive preparative isoelectric focusing steps in a matrix-free sucrose gradient. The MGL protein, but not the detergent, was concentrated and purified (see "Experimental Procedures"). The last two purification steps were gel exclusion chromatography and isoelectric focus-
ing with a pH 5–8 gradient, essentially according to the original procedure (see Figs. 2 and 5 in Ref. 5). Peak MGL activity fractions (Fig. 1) from the last isoelectric focusing step showed sufficient purity (>75%) to allow recovery of the 32.9-kDa MGL protein from SDS-PAGE for in-gel trypsinization and peptide sequencing.

The purified protein was cleaved with trypsin, and seven of the peptides obtained after HPLC separation were sequenced (Fig. 2). The Marathon cDNA amplification kit was used for PCR amplification of MGL sequences from mouse adipose tissue mRNA, with specific primers designed from the tryptic peptides of rat MGL. The largest fragment obtained was 519 bp long (the sense oligonucleotide derived from peptide 4 and the antisense oligonucleotide derived from peptide 3; Figs. 2 and 3).

The deduced amino acid sequence was found to contain peptides 1, 5, and 7 thus confirming the identity of the cDNA. Upon screening a mouse fat cell cDNA library with the 519-bp fragment as a probe, several positive clones were identified. The three largest inserts from these phage clones were subcloned into pBluescript SK and sequenced. The sequences were found to be overlapping, and together they represent the entire coding region. The nucleotide sequence and the predicted amino acid sequence of mouse MGL are presented in Fig. 3. The seven tryptic peptides of rat MGL were all identified in the mouse MGL sequence. The identity of these peptides to the deduced amino acid sequence of mouse MGL is 95%, indicating a high degree of conservation between rat and mouse MGL. The ATG (nucleotides 1–3) is suggested to be the translation initiation codon, since it is the first and only ATG in an open reading frame before the first identified tryptic peptide. In addition, the assignment of this ATG as the translation initiation codon is supported by some preliminary results from N-terminal sequencing.

![Amino acid sequence alignment of MGL with several related microbial proteins.](image)

| Protein                  | Species            | Accession Number |
|--------------------------|--------------------|------------------|
| PRXC_PSEPY               | Pseudomonas pyrogenes | PRXC_PSEPY       |
| PRXC_STRLI               | Streptomyces lividans | PRXC_STRLI       |
| ESTE_PSEFL               | Pseudomonas fluorescens | ESTE_PSEFL      |
| BPA1_STRAU               | S. aureofaciens     | BPA1_STRAU       |
| BPA2_STRAU               | S. aureofaciens     | BPA2_STRAU       |
| PLDB_ECOLI               | E. coli            | PLDB_ECOLI       |
| PLDB_HAININ              | H. influenzae       | PLDB_HAININ      |
| PRXC_PSEPY               | Pseudomonas pyrogenes | PRXC_PSEPY       |
| PRXC_STRLI               | Streptomyces lividans | PRXC_STRLI       |
| ESTE_PSEFL               | Pseudomonas fluorescens | ESTE_PSEFL      |
| BPA1_STRAU               | S. aureofaciens     | BPA1_STRAU       |
| BPA2_STRAU               | S. aureofaciens     | BPA2_STRAU       |

The hypothetical proteins deduced from GenBank entries are as follows. PseuPut, esterase from Pseudomonas putida (27); Cowpox, gene M5L from cowpox virus (accession number X94355); AraTha, lysophospholipase isolog from Arabidopsis thaliana (accession number U95973); MycGen, magnesium-chelatase homologue from Mycoplasma genitalium (accession number L43867).
His-269, -272, -284, and -292 mutated to Ala.

D239/243N

different constructs are denoted as follows.

values are calculated from three 60-mm dishes for each construct. The

is very similar to the molecular mass of 32.9

amino acids and to have a molecular weight of 33,218. This

mature mouse adipocyte MGL is predicted to be composed of

suming the same processing of the mouse and rat enzyme),

proteins.3 An amino acid sequence alignment for some of these

sequence identities between MGL and a number of microbial

the catalytic site serine (23). Data base searches revealed

the latter is found in many lipases, 70–100 amino acids N-terminal

before the monoglyceride-hydrolyzing enzyme.

lysophospholipase.

E. coli

Two lipase motifs were identified in the primary sequence,

active site serine motif GXSG and the HG dipeptide. The

latter is found in many lipases, 70–100 amino acids N-terminal

d of the catalytic site serine (23). Data base searches revealed

sequence identities between MGL and a number of microbial

proteins.3 An amino acid sequence alignment for some of these

proteins is shown in Fig. 4.

Transient Expression of MGL in COS Cells and Probing of

the Catalytic Triad by Site-directed Mutagenesis—The MGL

coding sequence was subcloned into the eukaryotic vector pCI-

neo for transient expression in COS cells. Homogenates of COS

cells transfected with the MGL/pCI-neo construct exhibited

sequencing of rat MGL, showing that proline is the N-terminal

residue, followed by glutamic acid (data not shown).

Based on the deduced amino acid sequence and the prelim-

inary assignment of proline as the N-terminal amino acid (as-

suming the same processing of the mouse and rat enzyme),

mature mouse adipocyte MGL is predicted to be composed of

302 amino acids and to have a molecular weight of 33,218. This

is very similar to the molecular mass of 32.9 ± 0.4 kDa for rat

adipocyte MGL, determined by SDS-PAGE (5).

Two lipase motifs were identified in the primary sequence,

the active site serine motif GXSG and the HG dipeptide. The

latter is found in many lipases, 70–100 amino acids N-terminal

d of the catalytic site serine (23). Data base searches revealed

sequence identities between MGL and a number of microbial

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Transient Expression of MGL in COS Cells and Probing of

the Catalytic Triad by Site-directed Mutagenesis—The MGL

coding sequence was subcloned into the eukaryotic vector pCI-

neo for transient expression in COS cells. Homogenates of COS

cells transfected with the MGL/pCI-neo construct exhibited

high levels of MO- and PNPB-hydrolyzing activity compared

with COS cells transfected with the pCI-neo vector alone (Fig.

5). As expected, MGL exhibited practically no catalytic activity

against a diglyceride, a triglyceride, and a cholesterol ester

substrate (<2% compared with MO-hydrolyzing activity; data

not shown).

The alignment shown in Fig. 4 suggests that the catalytic

triad of MGL is formed by Ser-122, Asp-239, and His-269. To

verify this triad, the three residues were individually mutated,

and the mutant proteins were expressed in COS cells. A

number of control mutations were analyzed in parallel. These

included all histidines present downstream from the active site

serine and Asp-243, which is highly conserved in the MGL

subfamily and in the lysophospholipases (Fig. 4). As shown in

Fig. 5, mutating Ser-122, Asp-239, or His-269 completely abol-

ished both the lipase and esterase activity of MGL, whereas all

of the other mutant proteins retained catalytic activity.

Tissue Distribution of MGL mRNA—A number of rat tissues,

including adipose tissue, adrenal gland, ovary, heart, brain,

spleen, lung, liver, skeletal muscle, kidney, and testis, were

analyzed for the presence of MGL mRNA by Northern blot

analysis (Fig. 6). A single mRNA transcript of ~4 kilobases was

identified in adipose tissue and all other tissues examined.

DISCUSSION

In the present study, we have isolated and characterized the
cDNA for mouse MGL by screening an adipocyte cDNA library
(Fig. 3). The deduced protein sequence for mature MGL is 302
amino acids long, corresponding to a molecular weight of
33,218. To our knowledge, this is the first described sequence of

a specific monoglyceride-hydrolyzing enzyme.

The closest relative to MGL found in the data base is a
hypothetical protein, encoded by the genome of cowpox virus
(GenBankTM accession number X94355). The sequence of this
protein shows more than 40% identity with MGL. Initial data
base searches with BLAST (15) revealed a number of bacterial
and yeast proteins that show between 20 and 25% sequence
identity with MGL at the amino acid level. These include an
esterase from Pseudomonas putida, a hypothetical 35.5-kDa
protein from Saccharomyces cerevisiae, one protein each from
Arabidopsis thaliana and Mycoplasma genitalium, and one
lysophospholipase each from Escherichia coli and Hemophilus
influenzae. Furthermore, the esterase from P. putida is, in fact,
more closely related to a family of haloperoxidases. Thus, MGL
shows a distant evolutionary relationship to esterases, lysop-

hospholipases, and haloperoxidases (Fig. 4). The three-dimen-

sional structure for one member of the haloperoxidase family,

the bromoperoxidase from Streptomyces aureofaciens

(BPA2_STRAU), has been solved by x-ray crystallography (24),
showing that these proteins have the αβ-hydrolase fold char-
acteristic for lipases and esterases (7). By comparing the amino acid sequence of the BPA2\_STRAU protein and MGL (Fig. 4), the secondary structure elements that constitute the α/β-hydrolase fold of MGL could be located in the primary sequence. When an alignment of MGL and the cowpox protein was used as the input data, all of these elements were correctly predicted by the PredictProtein PHD secondary structure prediction program (25). The information provided by Fig. 4 enabled us to build a partial three-dimensional model for MGL, by standard homology modeling techniques (not shown). In addition, the catalytic triad was identified, not only of MGL but also of all the related proteins. The results from the site-directed mutagenesis experiments (Fig. 5) were in complete agreement with the triad suggested by the alignment. These experiments were of particular relevance for the unambiguous identification of the aspartic acid of the triad, since the aspartic acid is not conserved in the hypothetical protein of the cowpox virus, which has an asparagine in that particular position (Fig. 4). Furthermore, the two lysophospholipases have a glutamic acid instead of an aspartic acid. This conserved substitution is commonly found among other families of lipases/esterases, e.g. the carboxylesterase B family (8), and only requires slight rearrangements of the side chains to allow the correct geometry of the triad. On the other hand, the presence of a highly conserved aspartic acid in the MGL group and in the lysophospholipases (Asp-243 in MGL, Figs. 3 and 4), raised the possibility that this aspartic acid in the MGL group and in the lysophospholipases was the residue involved in the catalytic triad. From a structural point of view, this would only mean a longer connecting loop from β7. However, as shown in Fig. 5, Asp-239 is essential for MGL activity, whereas Asp-243 is not.

From the alignment shown in Fig. 4 and from the known structures of BPA2\_STRAU (24) and other esterases and lipases, the structures of these proteins can be clearly divided into two conceptual modules: a central core harboring the essential elements of the α/β-hydrolase fold, including the catalytic machinery, and an external region located between strands β6 and β7 (shadowed in light gray in Fig. 4). The core module shows a degree of homology compatible with the maintenance of the α/β-hydrolase fold. On the contrary, the other module displays a high degree of variability among the different proteins. This modular division of the structure has provided an efficient way to generate a large superfamily of hydrolytic enzymes with a very broad substrate specificity.

The MGL transcript was observed in all tissues examined (Fig. 6), indicating that MGL functions as a widespread intracellular monoglyceride-hydrolyzing enzyme. An intriguing observation is that the MGL coding sequence only represents approximately 25% of the transcript length.

In conclusion, we have cloned and described the primary sequence of adipose tissue MGL, provided structural information based on sequence comparison with a distantly related family of enzymes, identified and probed the residues of the catalytic triad, and shown that MGL mRNA is constitutively expressed in the body. The described cDNA sequence of mouse MGL provides, for the first time, possibilities to perform structure-function relationship studies of this enzyme. Furthermore, after large scale expression and purification, structure determination should be feasible. This will hopefully provide further insight into the relationships between structure and substrate specificity.

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