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Characterization of cDNA encoding the mouse hepatic triglyceride lipase and expression by in vitro translation

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A cDNA coding for the mouse hepatic triglyceride lipase has been isolated from a mouse liver cDNA library. The nucleotide sequence of the cDNA shows an open reading frame encoding a polypeptide of 510 amino acids that is 91.5% and 86% homologous to rat and human hepatic lipase, respectively. The most drastic protein sequence divergence is found at the carboxyterminal end which was speculated to harbour one heparin-binding site. By in vitro translation of cRNA in the presence of pancreatic membranes the hepatic lipase was shown to be glycosylated and to have an electrophoretic mobility of 53 kDa.

Mouse hepatic triglyceride lipase; cDNA cloning; Amino acid composition; In vitro translation

1. INTRODUCTION

Hepatic triglyceride lipase (EC 3.1.1.34) functions in the metabolism of lipoproteins. The purified hepatic lipase hydrolyzes mono-, di-, triglycerides and phospholipids [1]. In vivo it acts on circulating lipoproteins and is presumably involved in the hydrolysis of phospholipids and triacylglycerides in high-density and very-low- density lipoproteins [2]. Furthermore, the enzyme appears to play a role in the metabolism of chylomicrons [3] and intermediate-density lipoproteins [2]. The hepatic triglyceride lipase (HL) is synthesized and secreted by parenchymal liver cells. In human and most vertebrates the HL enzyme is primarily bound to receptor molecules on endothelial liver cells and therefore the lipase activity in blood is low [4,5]. The HL enzyme can be rapidly released from the liver by heparin and therefore it is believed that the acceptor is a heparin-like polysaccharide [2]. In contrast to human and rat HL (hHL, rHL), the mouse HL (mHL) has a relatively low affinity for heparin-like polysaccharides and was shown to lack a high-affinity binding site [6]. The rHL and hHL was recently cloned and comparison of the corresponding primary sequences demonstrated that hHL and rHL are part of a multigene family [7,8]. Here we report on the cloning and sequence determination of the mouse hepatic triglyceride lipase (mHL), on the comparison of the derived amino acid sequence with the sequence of rat specific and human specific hepatic triglyceride lipase, and on the in vitro synthesis of mHL using the mHL cDNA.

The nucleotide sequence(s) presented here has (have) been submitted to the EMBL/GenBank database under the accession number no. X58426.

Abbreviation: h(m,r)HL, human (mouse, rat) hepatic triglyceride lipase

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2. MATERIALS AND METHODS

2.1. *cDNA library screening and sequencing*

A mouse liver cDNA λ-Zap library (Stratagene) was used as the source for DNA amplification with Taq polymerase (PCR) by using synthetic oligonucleotides. Oligonucleotides RHL24 (5'-CCCGGGTAAGACGAGAGACATGG3') and HL26 (5'-GGCTGGAAGGAGCCCCC-3') homologous to the 5'-end and to the middle part of rHL and hHL, respectively, were used for amplification of the 5'-part of the mHL cDNA, and oligonucleotides RHL27 (5'-GGGTCTCTTGACTCATC-3') and HL25 (5'-GGGGGCTCCTTCCAGCC-3') homologous to the 3'-end and to the middle part of rHL and hHL were used for amplification of the 3'-part of the mHL cDNA region. The PCR was run for 35 cycles (denaturation at 94°C for 1 min, annealing at 37°C for 2 min, extension at 72°C for 3 min) as described [9]. The two amplified fragments were cloned into the SK vector (Stratagene) for sequencing. To isolate full-length mHL cDNA, amplified fragments were radiolabeled with [α-^{32}P]dCTP by the multiprime DNA labeling system (Amersham) and used as probes for screening a mouse liver cDNA λ-Zap library (Strategene) as described [8]. Only partial mHL cDNAs were obtained. A full-length mHL cDNA which covers the complete sequence of mHL was therefore constructed using mHL cDNA fragments (isolated from the library) with a partial overlap. The fragments were used as templates in PCR (cycling times: 1 min at 94°C (denaturation), 2 min at 37°C (annealing) and 5 min at 72°C (extension) by using oligonucleotides RHL24 and poly dT(30). Fifty cycles were performed ending with an additional extension reaction for 10 min at 72°C.

The nucleotide sequence of mHL was determined by the dideoxy chain termination method [10] with the kit from United States Biochemical using [α-^{35}S]dATP and various synthetic oligonucleotides as primers.

2.2. *Transcription and translation in vitro*

A modified protocol of Melton et al. [11] was used for in vitro transcription of HL cDNA containing SP6 plasmids. The linearized plasmid (0.5–1 μm) purified by CsCl equilibrium centrifugation was incubated in 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 10 mM dithiothreitol, 4 mM spermidine, 0.5 mM of each ribonucleoside triphosphate, 0.14 mM $m^7G(5')ppp(5')G$ (Pharmacia), 30 U RNasin (Promega Biotec) and 5U of SP6 RNA polymerase (New England Biolabs) in a total volume of 50 μl for 1 h at 40°C. The template DNA was digested by RNase free DNase I (Boehringer Mannheim) and incubated for 10 min at 37°C. The RNA was extracted by phenol/chloroform/isoamylalcohol (25:24:1), precipitated with ethanol, dried and resuspended in water.

In vivo translation was carried out using the rabbit reticulocyte lysate (Promega Biotec and [^{35}S]methionine with or without the addition of pancreatic membranes according to the manufacturer's protocol. An aliquot was analyzed by separation in SDS-PAGE; the gels were soaked in 1 M sodium salicylate, dried and exposed to X-ray films.

3. RESULTS AND DISCUSSION

We have isolated a hepatic triglyceride lipase cDNA from a mouse (strain B6/CBA) liver λZap cDNA library. Oligonucleotides which represent conserved rat and human hepatic triglyceride lipase sequences were used as primers in PCR which was directly performed with λ phages of the cDNA library. Using the primers

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                                                                CCCGGGTAAGACGAGAC   -19
ATGGGAAATCCCCTCCAAATCTCCATTTTCCTGGTGTTCTGCATCTTTATCCAATCAAGTCCTTGTGGACAAGCCGTGGGAACAGAGCCC    90
m  g  n  p  l  q  i  s  i  f  l  v  f  c  i  f  i  q  s  s  a  c  G  Q  G  V  G  T  E  P     *8

TTTGGGAGAAGCCTTGGAGCTACTGAAGCTAGCAAGCCATTAAAGAAGCCAGAGACCAGATTCCTGCTCTTCCAAGATGAAAACGATCGC   180
F  G  R  S  L  G  A  T  E  A  S  K  P  L  K  K  P  E  T  R  F  L  L  F  Q  D  E  N  D  R     *38

CTGGGCTGTCGTCTCAGACCTCAGCACCCGGAAACACTGCAGGAGTGTGGCTTCAACAGCTCTCAGCCGCTTATCATGATCATCCACGGG   270
L  G  C  R  L  R  P  Q  H  P  E  T  L  Q  E  C  G  F  N  S  S  Q  P  L  I  M  I  I  H  G     *68

TGGTCGGTGGATGGCTTGCTAGAAAACTGGATCTGGAAGATAGTGAGTGCGCTGAAGTCCCGACAGTCCCAACCTGTGAATGTGGGGTTA   360
W  S  V  D  G  L  L  E  N  W  I  W  K  I  V  S  A  L  K  S  R  Q  S  Q  P  V  N  V  G  L     *98

GTGGACTGGATCTCCCTGGCATACCAGCACTACACCATTGCTGTTCAAAACACCCGTATTGTGGGCCAGGACGTGGCTGCTCTTCTCCTA   450
V  D  W  I  S  L  A  Y  Q  H  Y  T  I  A  V  Q  N  T  R  I  V  G  Q  D  V  A  A  L  L  L     *128

TGGCTGGAGGAATCTGCGAAGTTTTCTCGGAGCAAAGTTCACCTAATTGGGTACAGCCTGGGAGCGCACGTCTCAGGGTTCGCAGGCAGC   540
W  L  E  E  S  A  K  F  S  R  S  K  V  H  L  I  G  Y  S  L  G  A  H  V  S  G  F  A  G  S     *158

TCCATGGACGGGAAGAACAAGATTGGAAGAATCACAGGGCTGGACCCTGCGGGCCCTATGTTTGAGGGAACGTCCCCCAACGAGCGCCTT   630
S  M  D  G  K  N  K  I  G  R  I  T  G  L  D  P  A  G  P  M  F  E  G  T  S  P  N  E  R  L     *188

TCTCCTGATGATGCCAATTTTGTGGACGCCATTCATACCTTTACCAGGGAGCACATGGGCTTGAGTGTGGGCATCAAGCAGCCCATTGCC   720
S  P  D  D  A  N  F  V  D  A  I  H  T  F  T  R  E  H  M  G  L  S  V  G  I  K  Q  P  I  A     *218

CACTATGACTTCTACCCCAACGGGGGCTCCTTCCAGCCTGGCTGCCACTTCCTGGAACTCTACAAACACATTGCAGAGCATGGCCTAAAC   810
H  Y  D  F  Y  P  N  G  G  S  F  Q  P  G  C  H  F  L  E  L  Y  K  H  I  A  E  H  G  L  N     *248

GCCATAACCCAGACCATCAAATGTGCCCATGAGCGCTCCGTGCACCCCTTCATTGACTCCTTGCAACACAGTGACCTGCAGAGCATCGGC   900
A  I  T  Q  T  I  K  C  A  H  E  R  S  V  H  P  F  I  D  S  L  Q  H  S  D  L  Q  S  I  G     *278

TTCCAGTGCAGCGACATGGGCAGCTTCAGCCAAGGTCTATGCCTGAGCTGCAAAAAGGGCCGTTGCAACACTCTGGGTTATGACATCCGC   990
F  Q  C  S  D  M  G  S  F  S  Q  G  L  C  L  S  C  K  K  G  R  C  N  T  L  G  Y  D  I  R     *308

AAGGACCGGTCAGGCAAGACCAAGAGGCTCTTCCTCATCACGCGAGCCCAGTCTCCCTTCAAAGTTTATCATTACCAGTTCAAGATCCAG  1080
K  D  R  S  G  K  S  K  R  L  F  L  I  T  R  A  Q  S  P  F  K  V  Y  H  Y  Q  F  K  I  Q     *338

TTCATCAATCAAATTGAGAAGCCGGTAGAGCCTACTTTTACCATGTCGCTGCTGGGAACAAAAGAAGAAATAAAGAGAATTCCCATCACC  1170
F  I  N  Q  I  E  K  P  V  E  P  T  F  T  M  S  L  L  G  T  K  E  E  I  K  R  I  P  I  T     *368

CTGGGCGAAGGAATTACCAGCAATAAAACCTATTCCTTCCTTATCACACTGGACAAAGACATCGGCGAGTTGATCCTGCTCAAGTTCAAG  1260
L  G  E  G  I  T  S  N  K  T  Y  S  F  L  I  T  L  D  K  D  I  G  E  L  I  L  L  K  F  K     *398

TGGGAAAACAGTGCAGTGTGGGCCAATGTGTGGAACACAGTGCAGACCATCATGCTATGGGGCATAGAACCTCACCACTCTGGCCTCATT  1350
W  E  N  S  A  V  W  A  N  V  W  N  T  V  Q  T  I  M  L  W  G  I  E  P  H  H  S  G  L  I     *428

CTGAAGACCATCTGGGTCAAAGCTGGAGAGACGCAGCAAAGAATGACATTTTGCCCCGAAAATCTGGATGACCTCCAGCTTCACCCGAGC  1440
L  K  T  I  W  V  K  A  G  E  T  Q  Q  R  M  T  F  C  P  E  N  L  D  D  L  Q  L  H  P  S     *458

CAGGAGAAAGTCTTTGTGAACTGTGAAGTGAAGTCAAAAAGACTGACTGAATCGAAAGAGCAGATGAGTCAAGAGACCCATGCAAAAAAA  1530
Q  E  K  V  F  V  N  C  E  V  K  S  K  R  L  T  E  S  K  E  Q  M  S  Q  E  T  H  A  K  K     *488

TAAAGAAGTCTATTCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAA                                            1578
stop
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Fig. 1. Alignment of the nucleotide and deduced amino acid sequence for the mouse hepatic triglyceride lipase cDNA. Predicted amino acid residues are shown under the nucleotide sequence and numbered from the initiating glycine. The signal sequence is indicated by small letters. Nucleotides are numbered starting from the first nucleotide of putative ATG initiation codon. The putative processing/polyadenylation signal is marked by circles. Regions of mHL with sequence identity to hHL in which introns are presumably localized are underlined. The exact sites of intron positions in the corresponding human hepatic triglyceride lipase are indicated by an arrow.

RHL24 and HL26 a fragment of 777 bp (mHL-5′) was obtained coding for the 5′-region of mHL, and with primers RHL27 and HL25 a further fragment of 758 bp coding for the 3′-end of the mHL-coding region (mHL-3′# was obtained and both amplified fragments were sequenced. To obtain the sequence of the very carboxy-terminal end of the mHL cDNA, the ^{32}P-labeled mHL-3′ fragment was used as a probe for cDNA library screening by plaque hybridization. Thus, a cDNA clone of about 1200 bp (mHL-3′b) was isolated which encodes most of the coding region of mHL except the amino terminal end and the 3′-untranslated region including a putative polyadenylation signal and the poly(A) tail. In order to create a complete mHL cDNA fragment the mHL-3′-b and mHL-5′ fragments, which showed an overlap of 389 bp, were mixed, denatured, renatured and used as templates in a PCR assay. The RHL24 oligonucleotide and a poly(T) specific oligonucleotide were then used as primers. The resulting 1597 bp cDNA contained an open reading frame of 1533 nucleotides encoding a protein of 510 amino acids with a calculated molecular weight of 57.372 (Fig. 1). Comparison of the sequence of the coding region with that of hHL and rHL gene indicates a sequence homology of 86% and 91.5% on the protein level. Interestingly, sequences of the hHL exon/intron boundaries previously reported [12] are strictly conserved in the mHL sequence (Fig. 1). These data indicate that (i) all three enzymes are similar in protein structure, (ii) mHL is more closely related to rHL, and (iii) that mHL is probably also similar in genomic organization to hHL. Similar to the signal sequence described for rHL [7], hHL [8] and porcine pancreatic lipase [13], the mHL sequence from amino acid positions 1 to 22 refers to a putative hydrophobic leader sequence. Therefore the amino terminus of mature mHL begins probably at the glycine residue after the possible leader sequence as identified by protein sequencing of rHL [7]. The calculated isoelectric point of mature mHL is 8.14 and the estimated molecular weight is 54 958 which is similar to that determined experimentally by in vitro translation in presence of membranes (Fig. 3) or as expressed in vivo [14]. The mouse HL contains two potential N-linked glycosylation sites at Asn^{57} and Asn^{376}. In vitro translation of the mHL cRNA in the presence of pancreatic membranes revealed an electrophoretic mobility of mHL slightly higher than when synthesized without membranes (Fig. 3). As previously shown for rat mHL [15], a similar shift in electrophoretic mobility is due to glycosylation at two sites. Taking further into account that the potential sites of glycosylation are conserved in mHL and rHL [7,8], this suggests that mHL is also glycosylated at both sites. The highest protein sequence conservation is found in regions previously found important for structure or function of lipases. For example, three of the six cysteine disulfide bridges which are structurally important for porcine pancreatic lipase [16] are conserved in mHL,

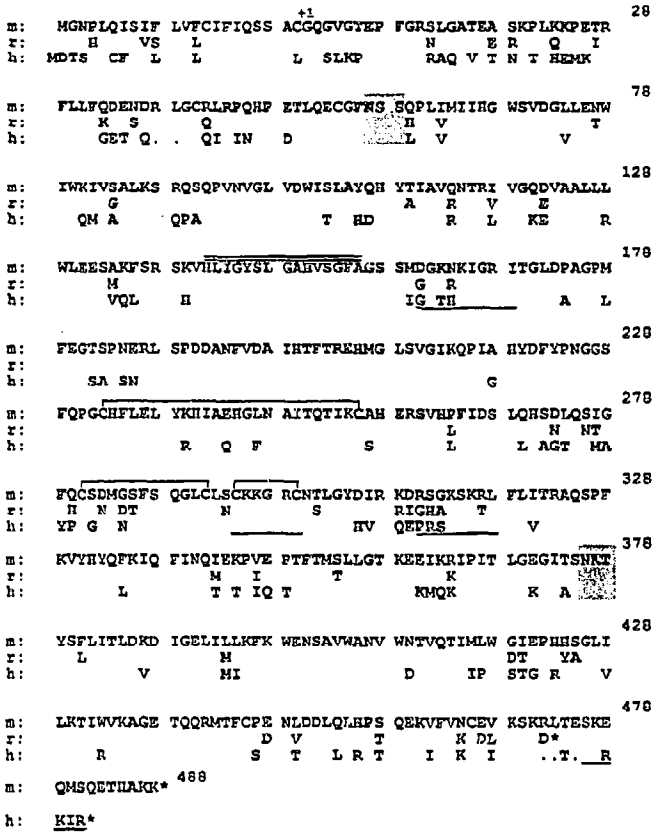

Fig. 2. Protein sequence comparison of mouse (m), rat (r) and human (h) hepatic triglyceride lipase. The amino acid positions refer to the mouse sequence. The interfacial binding site is double overlined. The disulfide bridges as determined for porcine pancreatic lipase are marked with arrow-bridges. The two glycosylation sites are marked by filled boxes. The four postulated heparin binding sites are underlined. Dots signify artificial gaps introduced for optimal aligning of the sequences.

rHL and hHL (Fig. 2). In addition, highly conserved are the sequences of the presumptive interfacial lipid binding site (amino acids 142–156) (Fig. 2) [8], the two presumed lipid-binding regions (amino acids 140–149, 260–269) and the long central homology region (amino

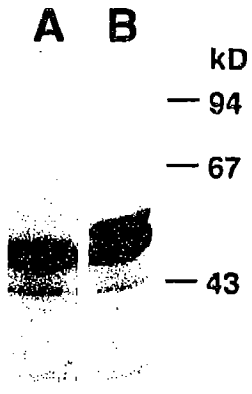

Fig. 3. Analysis of mHL mRNA translation in vitro. The mRNA was translated in the absence (lane A) or presence (lane B) of pancreatic microsomal membranes. M: molecular size marker, kd, kilodaltons.

acids 169–233) [7]. The motif Gly-Xaa-Ser-Xaa-Gly which is localized within the interfacial lipid binding site of mHL is also present in rHL and hHL. This motif is shared by serine proteases and esterases and it was shown that the central serine is required for the catalytic activity of rHL [17]. One of the four sequences (amino acids 161–169) that has been postulated to mediate heparin binding (underlined in Fig. 2) contains a mutation (residue 164) which is not in agreement with the heparin binding consensus sequence XBBBXXBX (B = basic residue) [18], one (amino acids 295–300) is conserved in all hepatic lipases, the third one (amino acids 311–318) contains mutations but they fit as well with the consensus sequence XBBBXXBX as those of human or rat, and only the fourth (amino acids 472–477 of hHL Ser-Lys-Arg-Lys-Ile-Arg) is not conserved due to a drastic difference to hHL and rHL which also differ strikingly (Fig. 2). It is therefore unlikely that the carboxyterminal sequences of hepatic lipases contribute to heparin binding.

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