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Primary structure of rat pancreatic lipase mRNA

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The sequence of a rat pancreatic lipase mRNA was determined. The data have been assigned the following accession number, X61925, in the EMBL data library. The total length of the messenger is 1531 nucleotides, plus a poly(A) stretch of about 60 nucleotides. A 72-nucleotides 5'-noncoding region is followed by a 1419-nucleotides open reading frame which encodes a protein of 473 amino acids, including the 17 amino acid signal peptide. The mature enzyme (456 residues) has 6 additional C-terminal amino acids, as compared with the amino acid sequence of pig (direct amino acid sequence), dog, man and rat isoenzyme from Genbank, M58369 (all deduced from the nucleotide sequence). A higher degree of homology exists between the amino acid sequence of rat mature enzyme with those of dog (88%), pig (75%) and man (75%) than with that of rat isolipase (74%).

Rat pancreas lipase; Nucleotide sequence

1. INTRODUCTION

Pancreatic lipase (triacylglycerol acyl hydrolase, EC 3.1.1.3) the physiological function of which is to hydrolyze dietary triacylglycerols in the duodenum, plays an important role in fat metabolism. The enzyme preferentially splits the esters of long-chain fatty acids at positions 1 and 3, producing mainly 2-monoacylglycerol and free fatty acids, and shows considerably higher activity against insoluble emulsified substrates than against soluble ones. The first step of the catalysis is an adsorption of the enzyme to the water-lipid interface [1]. The presence of various amphiphiles such as bile salts, by accumulating at the interface of emulsified particles, hinders lipase adsorption and completely abolishes lipase activity [2,3]. To overcome such an inhibition, pancreatic lipase must bind another protein, colipase, which, by adsorbing to the amphiphile covered interfaces, allows lipase to gain access to the substrate [4].

We have recently reported the nucleotide sequence of rat pancreatic colipase mRNA [5]. In this paper, we determined the nucleotide sequence of a rat pancreatic lipase cDNA and its deduced amino acid sequence (accession number in the EMBL Data Library, X61925). The comparison of the polypeptide chain sequence identity with another from rat (Genbank M58369), obtained in Dr. Mark E. Lowe's Laboratory (Washington University School of Medicine, St. Louis, USA), as well as with those from other species, will help, in conjunction with site-specific mutagenesis, in defining residues essential for interaction with colipase and lipids. We also report the partial nucleotide sequence of another cDNA clone, which is half-length and presents unambiguously some minor changes. This may reflect the cloning of 2 closely related, perhaps allelic, pancreatic lipase mRNAs.

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2. MATERIALS AND METHODS

2.1. *Materials*

[α-^{32}P]dCTP (>110 TBq/mmol) and [α-^{35}S]dATP (>37 TBq/mmol) were from Amersham Corp. (Les Ulis, France). The double-stranded deletion kit and sequencing kit were from Pharmacia (Saint-Quentin en Yvelines, France). The other reagents have already been described elsewhere [6].

2.2. *Library probing*

The construction of a rat pancreatic cDNA library in pUC9 has been reported [6]. Rat λgt 11 library was from Clontech (California, USA). cDNA inserts of interest from the phage library were subcloned into dephosphorylated and *Eco*R1-digested pUC18 plasmids for preparative growth. Rat pancreatic libraries were screened with a canine pancreatic lipase cDNA [7], radiolabelled by nick-translation [8].

2.3. *DNA sequence determination*

Plasmid DNA from positive clones were prepared and subjected to sequence analysis. The recombinant pUC9-lipase and pUC18-lipase cDNAs were cloned again in the corresponding plasmid in the other orientation after digestion by *Pst*I and *Eco*RI restriction enzymes, respectively. Partially deleted clones were obtained using the double-stranded nested deletion-kit from Pharmacia: recombinant pUC9-lipase plasmid was cut by *Bam*HI. Recessed 3' ends of DNA was then filled by Klenow fragment *E. coli* DNA polymerase in the presence of thiodeoxynucleotidetriphosphates. DNA was then digested by restriction enzyme *Hind*II. As far as the recombinant pUC18-lipase plasmid was concerned, it was double-digested by *Pst*I which generates 3'-protuding ends, and *Hind*II. Blunt ends of DNAs were unidirectionally digested by exonuclease III. Deleted plasmids were re-ligated and double-stranded DNA individually sequenced by the dideoxychain termination procedure [9,10] using a sequencing kit from Pharmacia. The entire sequence of the inserts were determined in this way, using

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AACAAGCACCTGGCTCCTGTGACCACACCAGTCACCTTAGGATCTGCATCTCCATCAGCTGAAAAAGCAGAGATGCTGACC
                                                                        MetLeuThr
                                                                        -17
CTCTGGACAGTCTCTCTTTTCCTACTGGGAGCAGCTCAGGGAAAAGAGGTTTGCTATGATAACCTTGGATGCTTTTCTGAC
LeuTrpThrValSerLeuPheLeuLeuGlyAlaAlaGlnGlyLysGluValCysTyrAspAsnLeuGlyCysPheSerAsp
                                              -1 1                              11
GCTGAGCCCTGGGCAGGGACAGCTATCAGGCCCCTCAAACTTCTCCCATGGAGCCCTGAGAAGATCAACACTCGCTTCCTG
AlaGluProTrpAlaGlyThrAlaIleArgProLeuLysLeuLeuProTrpSerProGluLysIleAsnThrArgPheLeu
                        21                                  31
CTGTACACCAATGAGAACCCAACTGCTTTTCAGACTCTCCAGCTTTCTGACCCATTGACCATTGGGGCCTCAAATTTTCAA
LeuTyrThrAsnGluAsnProThrAlaPheGlnThrLeuGlnLeuSerAspProLeuThrIleGlyAlaSerAsnPheGln
41                                  51                                  61
GTTGCCAGGAAGACTCGGTTTATCATCCATGGCTTCATAGACAAAGGAGAGGAAAACTGGGTGGTTGACATGTGCAAGAAC
ValAlaArgLysThrArgPheIleIleHisGlyPheIleAspLysGlyGluGluAsnTrpValValAspMetCysLysAsn
          71                                  81                                  91
ATGTTCCAAGTGGAGGAGGTGAACTGCATCTGTGTGGACTGGAAGAAAGGTTCTCAGACTACCTACACACAGGCTGCCAAC
MetPheGlnValGluGluValAsnCysIleCysValAspTrpLysLysGlySerGlnThrThrTyrThrGlnAlaAlaAsn
                    101                                 111                             121
AATGTGCGAGTAGTGGGTGCCCAGGTAGCTCAGATGATCGACATCCTTGTGAAAAACTACAGCTACTCGCCTTCCAAAGTC
AsnValArgValValGlyAlaGlnValAlaGlnMetIleAspIleLeuValLysAsnTyrSerTyrSerProSerLysVal
                              131                                 141
CACCTCATTGGCCACAGCCTAGGAGCCCACGTGGCCGGGGAAGCAGGAAGTCGGACTCCAGGTCTAGGCAGAATTACAGGA
HisLeuIleGlyHisSerLeuGlyAlaHisValAlaGlyGluAlaGlySerArgThrProGlyLeuGlyArgIleThrGly
      151                                 161                                 171
CTGGATCCTGTAGAAGCAAACTTCGAGGGCACTCCTGAAGAGGTCCGGCTTGACCCCTCGGATGCTGACTTTGTTGATGTG
LeuAspProValGluAlaAsnPheGluGlyThrProGluGluValArgLeuAspProSerAspAlaAspPheValAspVal
                181                                 191                             201
ATTCACACAGATGCAGCTCCCTTGATCCCGTTCTTGGGCTTCGGAACAAACCAAATGTCAGGGCACCTTGACTTCTTCCCC
IleHisThrAspAlaAlaProLeuIleProPheLeuGlyPheGlyThrAsnGlnMetSerGlyHisLeuAspPhePhePro
                          211                                 221
AACGGAGGACAGAGCATGCCCGGGTGCAAGAAGAATGCTCTGTCCCAGATTGTAGACATCGATGGCATCTGGTCAGGAACC
AsnGlyGlyGlnSerMetProGlyCysLysLysAsnAlaLeuSerGlnIleValAspIleAspGlyIleTrpSerGlyThr
   231                                 241                                 251
                                      AAA                                 GAC
CGGGACTTTGTGGCTTGTAACCACCTGAGAAGCTACAAGTACTACTTGGAGAGCATCCTTAACCCTGATGGGTTCGCTGCA
ArgAspPheValAlaCysAsnHisLeuArgSerTyrLysTyrTyrLeuGluSerIleLeuAsnProAspGlyPheAlaAla
              261                                 271                             281
TACCCCTGTGCTTCCTACAAGGACTTTGAGTCTAACAAATGCTTCCCCTGCCCAGATCAAGGCTGCCCACAGATGGGTCAC
TyrProCysAlaSerTyrLysAspPheGluSerAsnLysCysPheProCysProAspGlnGlyCysProGlnMetGlyHis
                          291                                 301
TATGCCGATAAGTTTGCCGGCAAGTCAGGTGACGAGCCACAGAAGTTCTTCTTGAACACAGGAGAAGCCAAGAACTTTGCA
TyrAlaAspLysPheAlaGlyLysSerGlyAspGluProGlnLysPhePheLeuAsnThrGlyGluAlaLysAsnPheAla
311                                 321                                 331
CGCTGGAGGTACCGTGTTTCCTTGATACTGTCTGGAAGAATGGTCACAGGGCAAGTCAAAGTGGCTCTGTTTGGAAGTAAG
ArgTrpArgTyrArgValSerLeuIleLeuSerGlyArgMetValThrGlyGlnValLysValAlaLeuPheGlySerLys
        341                                 351                                 361
ACAATACACGCC
GGCAATACACGCCAGTACGATATCTTCAGGGGAATTATCAAGCCTGGTGCTACACATTCCAGTGAGTTTGATGCCAAGCTC
GlyAsnThrArgGlnTyrAspIlePheArgGlyIleIleLysProGlyAlaThrHisSerSerGluPheAspAlaLysLeu
                    371                                 381                             391
GACGTGGGAACAATTGAGAAAGTCAAGTTTCTCTGGAACAATCAAGTGATAAACCCAAGCTTCCCCAAAGTGGGCGCAGCC
AspValGlyThrIleGluLysValLysPheLeuTrpAsnAsnGlnValIleAsnProSerPheProLysValGlyAlaAla
                              401                                 411
AAGATCACTGTGCAAAAGGGAGAGGAGCGGACGGAGTACAACTTCTGTAGTGAAGAGACCGTGAGAGAAGACACTCTGCTC
LysIleThrValGlnLysGlyGluGluArgThrGluTyrAsnPheCysSerGluGluThrValArgGluAspThrLeuLeu
      421                                 431                                 441
ACTCTCTTGCCTTGTGAAACCTCAGACACAGTGTGACCTCTTAGTGACACCAATAAAATCATCAGCTGCACTT(A)n
ThrLeuLeuProCysGluThrSerAspThrValSTOP
                    451
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of rat pancreatic lipase. The stop codon (TGA) and the polyadenylation signal (AATAAA) are underlined. (A)n indicates the poly(A) tract at the 3′ terminus of the mRNA. Signal peptide extends from residue −17 to residue −1. The site for proteolytic cleavage of the signal peptide between Gly −1 and Lys 1 is based on homology with the amino-terminal sequence of porcine lipase [16]. Residues numbers are indicated under the amino acid sequence. The differences found in the nucleotide sequence of the second partial clone are given above.

universal primer and reverse primer as sequencing primer for pUC9 and pUC18 clones, respectively. As unidirectional deleted DNAs were obtained, the recombinant cDNAs cloned in the other orientation were subjected to the same deletion procedure, to generate nested deletion libraries in the other direction and resulting clones were again individually sequenced. Nucleotide sequence of the entire inserts were thus determined from both strands.

3. RESULTS AND DISCUSSION

3.1. *Library screening*

Only 1 positive clone was obtained from the pUC9 cDNA library (about 1500 transformants) which was selected for sequence analysis. The pUC9-recombinant plasmid had an insert size of 692 nucleotides and encoded only the half terminal part of the protein (beginning at nucleotide 840, which corresponds to lysine 239). A few positive clones were prepared from the λgt11 library and only 1 (referred to as pUC18 recombinant clone) was long enough to include the 5′ untranslated region of mRNA. Further identity of these clones was also based on hybrid selection assays of mRNA, translation in the reticulum lysate system, and subsequent separation of the translated products by a one-dimensional gel electrophoresis. Only a few secretory proteins (about 20) have been shown to be expressed in exogenous pancreas [11]. The translation product had a characteristic position on the gel, corresponding to that of lipase.

3.2. *Nucleotide sequence*

The nucleotide sequence of rat lipase mRNA and the deduced amino acid sequence are shown in Fig. 1. pUC18 recombinant clone extends from nucleotide 1–1531, whereas pUC9 recombinant clone extends from nucleotide 840–1531. Both sequences were identical, except for 12 nucleotides, which represent less than 2% of base replacement: G(930), T(960) and G(1217–1227) were changed to A, C and ACAATACACGC, respectively, leading to the following amino acid residues: Lys(269), Asp(279) and Thr(365)-Ile-His-Ala(368), respectively (see Fig. 1 for changes in nucleotide sequence and Fig. 2 for amino acid replacement). The existence of 2 closely related lipase sequences may be explained by the cloning of 2 allelic genes. Up to now it was thought that there was a single lipase in rat pancreatic tissue. Indeed, when pancreatic juice or homogenized purified zymogen granules from Wistar rats were submitted to a 2-dimensional isoelectricfocusing/sodium dodecylsulfate gel electrophoresis, a single lipase spot could be visualized [11]. Moreover, Northern blot analysis of pancreatic mRNA shows a single hybridization band with nick-translated lipase probe of about 1600 nucleotides, suggesting, as far as the size is concerned, the presence of a single mRNA (data not shown). By contrast, several lipase isozymes were reported to be present in the pig [12], variability in the glycan chain being assumed to account for multiple forms of pig enzyme. The entire sequence of lipase mRNA which we describe here has 1531 nucleotides, plus a poly(A) tail of at least 60 nucleotides. A long 5′-noncoding region of 72 bases was found, which was significantly longer than the corresponding ones in dog (34 nucleotides [7]) or man (12 nucleotides [13,14]) and presented a low degree of identity with both 5′ untranslated segments (17 and 50% identity with dog and man sequences, respectively). The open reading frame extends from the initiation codon ATG at positions 73–75 to the termination codon TGA at positions 1492–1494. It is followed by a 37-nucleotide stretch which includes the consensus polyadenylation signal AATAAA located 16 nucleotides upstream from the poly(A) tail.

3.3. *Amino acid sequence*

The main amino acid differences between rat lipase sequence and the other reported sequences are summarized in the table. The lipase mRNA encodes a protein containing a signal peptide of 17 amino acids and a mature enzyme of 456 amino acids (mol. wt. of the processed molecule 57 798 kDa). The signal peptide terminates with a glycine at position −1, which is known to be the most frequent C-terminal residue in eukaryotic signal peptides [15]. The rat prepeptides are comparable in length and hydrophobicity to those of dog [7] and man [13,14]. The N-terminal residue of rat lipases, like that of dog and man enzymes, is a lysine, instead of a serine in the pig lipase [16]. The mature enzyme shares 65, 65, 66 and 82% identity with rat isoenzyme from Genbank, pig, man and dog lipases, respectively. If chemically similar amino acids are taken into account [17], the homology increases to 74, 75, 75 and 88%, respectively. Rat lipase has several additional residues, as compared with the already known sequences of pig, dog and man; Trp^{30} which is lacking in the pig sequence; Gln^{406} which is absent in the man enzyme, as well as in the rat isoenzyme from Genbank; Ser^{56} and Asn^{432} which is lacking in pig, man and rat (Genbank) lipases; as well as a C-terminal stretch of 6 residues (Glu^{451}–Val^{456}, which is unique in our rat sequence. It must be here emphasized that this -COOH extension has been found in all positive clones we isolated (1 from the pUC9-cDNA library and 4 from the λgt 11 library) and that the nucleotide sequence has been performed in both directions, thus eliminating sequencing errors (see Fig. 3 for nucleotide sequence). The high degree of identity of the dog cDNA probe with our rat sequence at the amino acid level (82%), as compared with that of rat isoenzyme sequence from Genbank (65%) certainly explains that, when screening rat cDNA libraries under rather stringent conditions with the dog probe, we isolated only 2 very similar forms of lipase. Two deletions relative to pig, man and rat isoenzyme from Genbank sequences are observed in ours from rat; an isoleucine between residues 170 and 171 and a tyrosine between residues 403 and 404. 13 Cys residues are conserved in

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                          1                   21                  41
rat 1  MLTLWTVSLFLLGAAQGKEVCYDNLGCFSDAEPWAGTAIRPLKLLPWSPEKINTRFLLYTNENPTA
rat 3    M    FAV-     VA      F K       DA  S  ID     A      AQ             QDN

                  61                  81                  101
rat 1  FQTLQLSDPLTIGASNFQVARKTRFIIHGFIDKGEENWVVDMCKNMFQVEEVNCICVDWKKGSQTT
rat 3  Y KI-T  ASS RN    KTN     I              LS        K  S          G  RA

           121                 141                 161
rat 1  YTQAANNVRVVGAQVAQMIDILVKNYSYSPSKVHLIGHSLGAHVAGEAGSRTPG-LGRITGLDPVE
rat 3      TQ        E  LLVNV KSDLGHP DN          S        K  F AI        AA

       181                 201                 221                 241
rat 1  ANFEGTPEEVRLDPSDADFVDVIHTDAAPLIPFLGFGTNQMSGHLDFFPNGGQSMPGCKKNALSQI
rat 3  PY Q           T  Q   A       I  N    MS TV           ME    Q  I

                  261                 281                 301
rat 1  VDIDGIWSGTRDFVACNHLRSYKYYLESILNPDGFAAYPCASYKDFESNKCFPCPDQGCPQMGHYA
rat 3         E     A           TD  V  T  SGFS S  NV SA      GSE

               321                 341                 361
rat 1  DKFAGKSGDEPQKFFLNTGEAKNFARWRYRVSLILSGRMVTGQVKVALFGSKGNTRQYDIFRGIIK
rat 2                                                    TIHA
rat 3    YP  TKELY   Y    DKS       Q TVT   QK   HIL S   NG  SK  EV K SLH

         381                 401                 421                 441
rat 1  PGATHSSEFDAKLDVGTIEKVKFLWNNQVINPSFPKVGAAKITVQKGEERTEYNFCSEETVREDTL
rat 3    D  VK   SDM   DLQ    I Y N    TL     SR S ERNDG V-F    QD     V


rat 1  LTLLPCETSDTV*
rat 3     SAC*
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Fig. 2. Amino acid sequences for rat pancreatic lipases. The complete sequence which was obtained from the λgt11 clone is given in the upper line (referred to as rat 1). The data have been assigned the following accession number in the EMBL Data Library: X61925. Only the minor changes, occurring between residue numbers 365 and 368 of the partial clone, derived from the pUC 9-cDNA library, are indicated (rat 2). Rat 3 sequence was reported in Genbank (M58369) and was realized in Dr. Mark E. Lowe's Laboratory (Department of Pediatrics, Washington University School of Medicine, St. Louis, MO 63114, USA). The differences found in the sequences are given. Residue numbers are indicated above the amino acid sequence. *Shows the last amino acid of the protein.

the rat sequences. Rat pancreatic lipases have the potential to form the 6 disulfide bridges present in the pig protein [18]. Residue Cys^{181} in pig and man lipase sequences, which is not involved in a disulfide bridge, is lacking in dog and both rat sequences, and replaced by an Asp in our clone or a Tyr in the other rat sequence (position 182 in the rat sequence). Although this SH group has been shown to be the more reactive SH group with *N*-ethylmaleimide of the pig enzyme, it is not essential for lipase activity [19]. The deduced amino acid sequences of rat lipases contain all the residues which have been demonstrated to be important in the catalytic

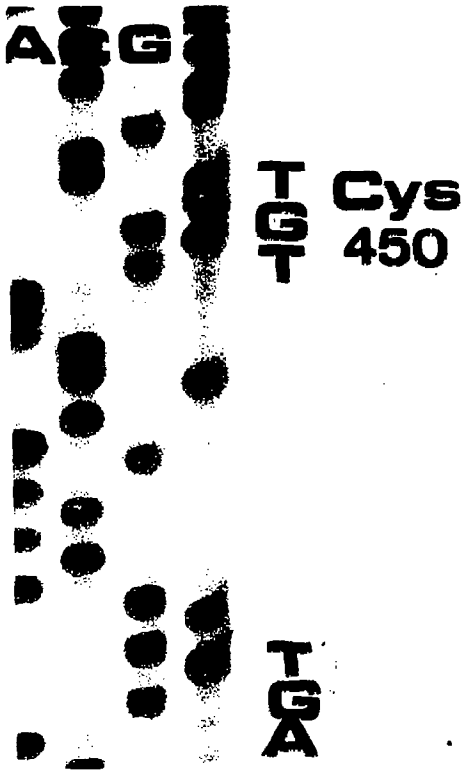

Fig. 3. Nucleotide sequence of the 3' end of the rat lipase mRNA corresponding to the COOH-terminal extension. Cys-450 in the amino acid sequence is indicated (TGT codon), as well as the stop codon (TGA).

activity or in the binding of the substrate. The hydrophobic sequence IGHSLG is conserved. This region contains the essential Ser^{152} (which corresponds to residue 154 in the rat sequence), the chemical modification of which results in the loss of enzymatic activity [20,21]. This residue, stoichiometrically labeled with the organophosphorus reagent E 600 (micellar diethyl *p*-nitrophenyl phosphate), was shown to be involved in catalysis since the binding of E 600-modified pancreatic lipase to lipid/water interface was comparable to that of native lipase [22]. It has been demonstrated from the crystallization data of human pancreatic enzyme [14] that the side chain of Ser^{154} was hydrogen-bonded to His^{264}, which made another hydrogen bond with Asp^{177}. The residues constituting the triad Asp-His-Ser are found in the sequence of rat lipase, as well as in those of pig, dog and man. This triad is covered by a surface loop or 'lid' [14,23], which has been suggested to move during interfacial activation [14]. This assumption has recently been directly supported by the crystal structure determination of a complex of a triacyl glycerol lipase from the fungus, *Rhizomucor miehei*, with the enzyme inhibitor *n*-hexylphosphonate ethyl ester, where the active site is exposed by the movement of the helical lid [24]. Other amino acids involved in pig lipase activity are present in rat pancreatic lipases such as one of the histidines 75 or 156 (positions 77 and 158 in the rat sequence), the ethoxyformylation of which results in the loss of activity toward triacylglycerol hydrolysis [26]. His^{354} of the acylation site of pig enzyme (corresponding to residue 355 in rat lipase), which reacts rapidly with ethoxyformic anhydride and permits the transient formation of an acyl lipase derivative [25] and Lys^{373} (corresponding to residue 374 in the rat, which is the first lysine in the pig enzyme to become acetylated by *p*-nitrophenylacetate and is responsible for colipase binding [26] are replaced by a glutamine and an arginine, respectively, in our sequence. Asn^{166}, which had been shown to be modified by the addition of a short glycan chain in porcine pancreatic lipase [16], is replaced by a proline in the rat and dog sequences and phenylalanine in rat isoenzyme sequence from Genbank (equivalent position, 168 in rat sequence). Two other potential *N*-glycosylation sites exist in the rat enzyme; Asn^{140}-Tyr-Ser and Asn^{409}-Pro-Ser.

In conclusion, the rat pancreatic lipase clone we isolated is more closely related to dog pancreatic lipase as

Table I

Summary of the main amino acid differences between rat lipase sequence and the other reported sequences

	Residue number*												
	1	30	56	140	168	between 170–171	182	355	374	between 403–404	406	432	451–456
clone 1**	K	W	S	N	P	del	N	Q	R	del	Q	N	ETSDTV
clone 2***								Q	R	del	Q	N	ETSDTV
clone 3****	K	W	del	D	F	I	Y	H	K	Y	del	del	del
Pig [16]	S	del	del	S	N	I	C	H	K	Y	N	del	del
Dog [7]	K	W	S	N	P	del	S	Q	K	del	N	S	del
Human [13,14]	K	W	del	A	N	I	C	H	K	Y	del	del	del

*Amino acid numbering corresponds to that derived from our rat lipase full-length clone.
**full-length cDNA isolated by us from the λgt11 cDNA library.
***partial cDNA isolated from the pUC9 cDNA library.
****unpublished sequence from Dr. M.E. Lowe's Laboratory (St Louis, USA), reported in Genbank (M58369).
del, deletion.

regards the position of the deleted and inserted amino acids as well as amino acid sequence. We have isolated another cDNA clone coding for pancreatic lipase with only minor changes in the partial sequence which may correspond to an allelic form of the gene. The presence of 6 additional residues in the C-terminal part of the protein may suggest that this part of the protein is not essential for lipolytic activity. Another unpublished cDNA sequence for rat pancreatic lipase is present in Genbank (M58369) with only 65% identity at the amino acid level with the presently reported sequence. Most of the changes are, however, conservative. These data provide strong evidence that at least 2 genes for pancreatic lipase are present in the rat. The full length cDNA for rat lipase reported here, and a full length cDNA for rat pancreatic colipase isolated recently by us [5], provide the ability to test the function of both pancreatic lipase and colipase by site-directed mutagenesis.

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