Molecular Cloning of the cDNA for Rat Hepatic, Bile Salt–Dependent Cholesteryl Ester/Retinyl Ester Hydrolase Demonstrates Identity with Pancreatic Carboxylester Lipase (44127)

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Abstract. **Rat liver homogenates contain a neutral lipid ester hydrolase that requires millimolar concentrations of bile salts for maximal activity in catalyzing the hydrolysis of cholesteryl esters and retinyl esters *in vitro*. Previous studies have demonstrated that this hepatic hydrolase resembles rat pancreatic carboxylester lipase because it reacts with a specific pancreatic carboxylester lipase antibody and the eight N-terminal amino acids of the hepatic protein are identical to those of the pancreatic enzyme. Nonetheless, the exact molecular relationship between the hepatic and pancreatic enzymes is unclear. In the present study, a rat hepatic cDNA encoding the enzyme was cloned. Sequence analysis demonstrated that this cDNA corresponds to the full-length mature pancreatic carboxylester lipase (EC# 3.1.1.13). In individual animals the hepatic and pancreatic cDNA sequences were identical. However, among rats there were sequence variations, suggesting a polymorphic nature for this rat gene.** [P.S.E.B.M. 1997, Vol 215]

Rat liver contains and secretes a bile salt–dependent neutral lipid ester hydrolase that shows a broad substrate range, including cholesteryl esters and retinyl esters. The enzyme requires millimolar concentrations of trihydroxy bile salts for activity in bulk assay systems. These properties are also characteristic of pancreatic carboxylester lipase (1–4). Indeed, much recent work on the hepatic enzyme has focused on the possibility that most or all of this activity is due to carboxylester lipase, an enzyme that has been purified from the pancreata and milks of several species (5). For example, we and others have previously shown (6–11) that rat liver contains and secretes an enzymatic activity indistinguishable by immunological tests and by amino-terminal protein sequencing from that of the neutral pancreatic bile salt–dependent carboxylester lipase (CEL, EC# 3.1.1.13).

Full-length cDNA sequences for rat pancreatic CEL have been reported (12, 13). Although some reports have also shown a hepatic mRNA of the expected length which hybridizes to pancreatic CEL cDNA (8, 12), it is not certain that the liver contains an enzyme *identical* to pancreatic CEL based on at least two considerations: not all investigators have found a rat hepatic mRNA species that hybridizes to a pancreatic CEL cDNA probe (14), and the hepatic enzyme has a much lower specific activity than does the pancreatic enzyme, as noted by Wang and Hartsuck (5).

Thus, it is possible that the pancreatic and hepatic enzymes are related but not identical. To understand the molecular nature of the hepatic enzyme, its relationship to the pancreatic enzyme needs to be established. In this report, we conclusively demonstrate by cDNA cloning the presence in rat liver of the same enzyme mRNA as the mature pancre-

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        10         20         30         40         50         60
GCAAAGTTGGGTGCTGTGTACACAGAAGGCGGTTTTGTGGAGGGCGTCAACAAGAAACTC
AlaLysLeuGlyAlaValTyrThrGluGlyGlyPheValGluGlyValAsnLysLysLeu
 *
        70         80         90        100        110        120
AGTCTCTTGGGTGGTGACTCTGTTGACATCTTCAAGGGCATCCCCTTTGCTACCGCCAAG
SerLeuLeuGlyGlyAspSerValAspIlePheLysGlyIleProPheAlaThrAlaLys

       130        140        150        160        170        180
ACCCTGGAGAATCCTCAGCGTCACCCTGGCTGGCAAGGGACACTGAAGGCTACAGACTTC
ThyLeuGluAsnProGlnArgHisProGlyTrpGlnGlyThrLeuLysAlaThrAspPhe

       190        200        210        220        230        240
AAGAAACGATGTCTACAAGCCACCATCACCCAGGATGATACCTATGGGCAAGAAGACTGC
LysLysArgCysLeuGlnAlaThrIleThrGlnAspAspThrTyrGlyGlnGluAspCys

       250        260        270        280        290        300
CTCTATCTCAACATCTGGGTCCCTCAGGGCAGGAAGCAAGTGTCTCATGACCTGCCTGTG
LeuTyrLeuAsnIleTrpValProGlnGlyArgLysGlnValSerHisAspLeuProVal

       310        320        330        340        350        360
ATAGTCTGGATCTATGGAGGTGCCTTCCTCATGGGGTCTGGCCAGGGAGCCAATTTTCTC
IleValTrpIleTyrGlyGlyAlaPheLeuMetGlySerGlyGlnGlyAlaAsnPheLeu

       370        380        390        400        410        420
AAGAATTACCTGTATGATGGGGAAGAGATCGCCACTAGAGGCAATGTCATTGTGGTCACC
LysAsnTyrLeuTyrAspGlyGluGluIleAlaThrArgGlyAsnValIleValValThr

       430        440        450        460        470        480
TTCAACTACCGTGTCGGACCCTTGGGTTTCCTTAGCACCGGAGATGCTAACCTTCCAGGT
PheAsnTyrArgValGlyProLeuGlyPheLeuSerThrGlyAspAlaAsnLeuProGly

       490        500        510        520        530        540
AACTTTGGACTTCGAGATCAGCACATGGCTATTGCCTGGGTGAAGAGGAACATTGCAGCC
AsnPheGlyLeuArgAspGlnHisMetAlaIleAlaTrpValLysArgAsnIleAlaAla

       550        560        570        580        590        600
TTTGGAGGAGACCCCGATAACATCACCATCTTTGGGGAATCTGCTGGAGCTGCCAGTGTC
PheGlyGlyAspProAspAsnIleThrIlePheGlyGluSerAlaGlyAlaAlaSerVal

       610        620        630        640        650        660
TCTCTGCAGACCCTCTCCCCATACAACAAGGGCCTCATCCGGCGAGCCATCAGTCAGAGT
SerLeuGlnThrLeuSerProTyrAsnLysGlyLeuIleArgArgAlaIleSerGlnSer

       670        680        690        700        710        720
GGTGTGGCACTGAGCCCCTGGGCCATCCAGGAGAATCCACTTTTCTGGGCCAAAACGATC
GlyValAlaLeuSerProTrpAlaIleGlnGluAsnProLeuPheTrpAlaLysThrIle

       730        740        750        760        770        780
GCTAAGAAGGTGGGATGCCCCACAGAGGATACCGCCAAGATGGCTGGGTGTCTGAAGATC
AlaLysLysValGlyCysProThrGluAspThrAlaLysMetAlaGlyCysLeuLysIle

       790        800        810        820        830        840
ACAGATCCCCGAGCCTTGACACTGGCCTACAGGTTGCCCTTGAAAAGCCAGGAGTACCCC
ThrAspProArgAlaLeuThrLeuAlaTyrArgLeuProLeuLysSerGlnGluTyrPro

       850        860        870        880        890        900
ATTGTGCACTACCTGGCCTTCATCCCTGTCGTCGATGGTGACTTCATTCCTGATGATCCC
IleValHisTyrLeuAlaPheIleProValValAspGlyASpPheIleProAspAspPro

       910        920        930        940        950        960
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Figure 1. Nucleotide and deduced amino acid sequence of rat hepatic CEL. The regions in the translated sequence used for the design of PCR primers are underlined once. The poly-adenylation signal is underlined twice. The translational stop codon is labeled "End." Nucleotide position 1 corresponds to the beginning of the first codon (*) of mature rat pancreatic CEL (12, 13). Note the eight extra nucleotides (positions 1974–1981) that follow the polyadenylation site in the rat hepatic enzyme sequence that are not present in the published sequences for the pancreatic enzyme.

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ATCAACCTGTACGACAACGCTGCTGACATTGACTACTTAGCGGGTATTAATGACATGGAT
IleAsnLeuTyrAspAsnAlaAlaAspIleAspTyrLeuAlaGlyIleAsnAspMetAsp

       970        980        990       1000       1010       1020
GGCCACCTGTTTGCTACAGTTGACGTGCCCGCCATCGACAAGGCCAAGCAGGATGTCACA
GlyHisLeuPheAlaThrValAspValProAlaIleAspLysAlaLysGlnAspValThr

      1030       1040       1050       1060       1070       1080
GAGGAGGACTTCTACAGGCTAGTCAGTGGACACACTGTCGCCAAGGGGCTTAAAGGCACC
GluGluAspPheTyrArgLeuValSerGlyHisThrValAlaLysGlyLeuLysGlyThr

      1090       1100       1110       1120       1130       1140
CAAGCCACCTTTGACATCTACACTTGTGCCTGGGCCCAGGACCCGTCCCAGGAGAACATG
GlnAlaThrPheAspIleTyrThrCysAlaTrpAlaGlnAspProSerGlnGluAsnMet

      1150       1160       1170       1180       1190       1200
AAGAAGACAGTGGTGGCCTTTGAGACTGACATACTCTTCCTGATCCCCACAGAGATGGCT
LysLysThrValValAlaPheGluThrAspIleLeuPheLeuIleProThrGluMetAla

      1210       1220       1230       1240       1250       1260
CTGGCCCAGCACAGAGCCCATGCCAAGAGTGCCAAGACCTACTCTTACCTGTTTTCCCAC
LeuAlaGlnHisArgAlaHisAlaLysSerAlaLysThrTyrSerTyrLeuPheSerHis

      1270       1280       1290       1300       1310       1320
CCTTCACGAATGCCTATCTACCCAAAATGGATGGGGGCAGACCACGCTGATGACCTCCAG
ProSerArgMetProIleTyrProLysTrpMetGlyAlaAspHisAlaAspAspLeuGln

      1330       1340       1350       1360       1370       1380
TACGTCTTTGGGAAGCCCTTTGCCACCCCACTGGGCTACCGGGCCCAAGACAGGACTGTC
TyrValPheGlyLysProPheAlaThrProLeuGlyTyrArgAlaGlnAspArgThrVal

      1390       1400       1410       1420       1430       1440
TCCAAGGCCATGATTGCCTACTGGACCAACTTTGCCAAGAGTGGGGACCCCAACATGGGC
SerLysAlaMetIleAlaTyrTrpThrAsnPheAlaLysSerGlyAspProAsnMetGly

      1450       1460       1470       1480       1490       1500
AACTCACCCGTGCCCACACACTGGTACCCTTATACCATGGAGAATGGTAACTACCTGGAC
AsnSerProValProThrHisTrpTyrProTyrThrMetGluAsnGlyAsnTyrLeuAsp

      1510       1520       1530       1540       1550       1560
ATCAATAAGAAAATAACCAGCACCTCCATGAAGGAGCACCTAAGGGAAAAGTTCCTCAAG
IleAsnLysLysIleThrSerThrSerMerLysGluHisLeuArgGluLysPheLeuLys

      1570       1580       1590       1600       1610       1620
TTCTGGGCTGTGACATTCGAGATGCTGCCCACTGTGGTTGGTGACCACACTCCCCCTGAG
PheTrpAlaValThrPheGluMetLeuProThrValValGlyAspHisThrProProGlu

      1630       1640       1650       1660       1670       1680
GATGACTCAGAGGCTGCCCCCGTCCCACCTACAGACGACTCCCAGGTTGTTCCTGTCCCA
AspAspSerGluAlaAlaProValProProThrAspAspSerGlnValValProValPro

      1690       1700       1710       1720       1730       1740
CCTACAGATGACTCTCAGACAACACCGGTGCCCCCAACAGACAACTCTCAGGCTGGTGAC
ProThrAspAspSerGlnThrThrProValProProThrAspAsnSerGlnAlaGlyAsp

      1750       1760       1770       1780       1790       1800
TCTGTGGAGGCTCAGATGCCTGTCGCCATTGGCTTCTAAAGTCCTATAAACCGGGGCTAG
SerValGluAlaGlnMetProValAlaIleGlyPheEnd

      1810       1820       1830       1840       1850       1860
AGATGGCTCAGGAGCTAAGAGCTCTTCTTCCACTGTTCTTCTGAAGGTCCTGTGTTCAAT

      1870       1880       1890       1900       1910       1920
TCCCAGCACCCACATTGCTGCTTACAGCTGTCTGATTCCGTCTACTGGTGTGCAGATGTA

      1930       1940       1950       1960       1970       1980
CACGCAGACAAAGCGCCTATATATAGAAAGTTCATACATAAATAAAGTCTTATAAGCCTC

      1990
GAAAAAAAAAAAAAAAAA
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Figure 1. Continued.

atic CEL and extend this result by showing that among individual rats, CEL is polymorphic.

Materials and Methods

The cDNA of rat hepatic CEL was cloned by rapid amplification of cDNA ends (15). Poly (A^+) mRNA from adult male Lewis rat liver or pancreas was isolated as previously described (16) and was reverse-transcribed in the presence of oligo (dT16) with an adapter and subsequently amplified by polymerase chain reaction (PCR). A previously published sequence of rat pancreatic CEL cDNA (12) was used to design PCR primers. Primers were chosen from either a region known to be identical between the pancreatic and hepatic enzymes (primer B; the 5′ end, first eight codons [9]) or a region highly conserved (primers C and D) among pancreatic CELs cloned from different animal sources (cow, human, rat; reviewed in Ref. 5). The sequences of the primers are as follows (small letters indicate restriction sites): dT16-adapter, 5′-GCGTCGAGAAGTCCgaattcTTTTTTTTTTTTTTT-3′ (contains *Eco*RI site); primer A, 5′-GCGTCGAGAAGCTTGAAT-3′ (adapter); primer B, 5′-ACTggatccGCAAAGTTGGGTGCTGTG-3′ (contains *Bam*HI site and nucleotides 1–18, Fig. 1); primer C, 5′-GTAgaattcCTGGAGGTCATCAGCGTGGTC-3′ (complementary to nucleotides 1299–1319, Fig. 1); primer D, 5′-ATTggatccGACCACGCTGATGACCTCCAG-3′ (contains *Bam*HI site and nucleotides 1299–1319, Fig. 1).

PCR products were analyzed by electrophoresis, excised from the gel, and purified. Purified DNA was either directly sequenced or digested with *Eco*RI and *Bam*HI, subcloned into plasmid vector pGEM-4Z (Promega Corp., Madison, WI), and then sequenced. For plasmid inserts, sequencing of both DNA strands was done by the dideoxy NTP chain termination method (17). When direct sequencing of PCR products was performed, the *fmol* DNA Sequencing System (Promega) was used with ^{33}P end-labeled primers. The sequencing ladder was separated on a 6% polyacrylamide/8.3 *M* urea sequencing gel which was dried and exposed to X-ray film. The sequences were manually read and then analyzed by the Genepro Program, Version 5.00 (Riverside Scientific Enterprises, Riverside, CA).

Results and Discussion

Two of the overlapping PCR clones, pCELX1 (between primers B and C, 1.3 kb in size) and pCELX2 (between primers A and D, 0.7 kb in size), yielded the cDNA sequence corresponding to the N-terminal codon of the mature pancreatic protein through the poly (A^+) tail. A cDNA starting with the N-terminal codon was expected because the primer B used was designed from the eight known N-terminal amino acids. The complete nucleotide sequence and deduced amino acid sequence of the open reading frame for rat hepatic CEL are shown in Figure 1. The cDNA sequence is 1998 bases in length and encodes a protein of 592 amino acids. This cDNA was compared to those of two published rat pancreatic CELs (12, 13). The sequence of the hepatic CEL cDNA was substantially the same as the two published sequences; however, differences between the hepatic and pancreatic forms of CEL were observed in twenty-one nucleotides, resulting in a change of eight amino acids from those of Ref. 12 (Table I). The other nucleotide differences among the three cDNA sequences were observed at the 3′-untranslated region of the mRNA. The hepatic CEL also contained an extra eight

Table I. Comparison of Rat Hepatic CEL cDNA Nucleotide and Deduced Amino Acid Sequences with Published Rat Pancreas CEL Sequences (12, 13)

Nucleotide (amino acid)	Rat pancreas (12)	Rat pancreas (13)	Rat liver (Fig. 1)
303	AT*G*	AT*G*	AT*A*
(101)	Met	Met	**Ile**
590,596	G*C*T GCC A*G*T	G*G*T GCC A*T*T	G*C*T GCC A*G*T
(197,199)	Ala Ala Ser	**Gly** Ala **Ile**	Ala Ala Ser
1105,1108	*GAG TCC*	*GAG TCC*	*TGT GCC*
(369,370)	Glu Ser	Glu Ser	**Cys Ala**
1478	A*C*G	A*T*G	A*T*G
(493)	**Thr**	Met	Met
1667,1670	G*G*T G*G*T	G*G*T G*G*T	G*T*T G*T*T
(556,557)	Gly Gly	Gly Gly	**Val Val**
1763,1765	G*GT CCC*	G*GT CCC*	G*TC GCC*
(588,589)	Gly Pro	Gly Pro	**Val Ala**
1880	GCT*G*CTT	GCT*T*CTT	GCT*G*CTT
	Untranslated	Untranslated	Untranslated
1950	AAA*A*TTC	AAA*A*TTC	AAA*G*TTC
	Untranslated	Untranslated	Untranslated
1974–	AA_N	AA_N	*AAGCCTCG*AA_N
	Untranslated	Untranslated	Untranslated

Note. Amino acid changes are in bold.

Table II. Comparison of Rat Hepatic to Pancreatic cDNA Sequences

Nucleotide (amino acid)	Rat		Rat liver (Fig. 1)
	Pancreas	Liver	
303	ATG	ATG	ATA
(101)	Met	Met	**Ile**
1105,1108	*GAG TCC*	*GAG TCC*	*TGT GCC*
(369,370)	Glu Ser	Glu Ser	**Cys Ala**
1950	AAAATTC	AAAATTC	AAAGTTC
	Untranslated		Untranslated
1974–	$AAGCTGAA_N$	$AAGCTGAA_N$	$AAGCCTCGAA_N$
	Untranslated		Untranslated

Note. Information in the right-hand column is based on Figure 1. The sequence data in the middle two columns were derived from pancreas and liver obtained from an additional animal. Amino acid differences are in bold.

nucleotides just before the poly (A^+) tail. It is unlikely that these differences were due to sequencing errors, because in cases of discrepancies both strands were sequenced to confirm the differences. To demonstrate definitively the identity of the hepatic and pancreatic cDNAs and to demonstrate that the differences summarized in Table I represented polymorphisms among the three individual animals (the two animals used in Refs. 12 and 13 and the one used here), sequence analysis of the regions described in Table I was performed on cDNA prepared from mRNAs isolated from pancreatic and hepatic tissues from another Lewis rat. As shown in Table II, there were also variations in the hepatic cDNA isolated from this rat compared with the Figure 1 cDNA. In contrast, the sequence of the companion pancreatic cDNA at these positions was exactly the same as the hepatic cDNA.

Cloning of a cDNA species from rat liver mRNA that has the same sequence as mature pancreatic CEL confirms the identity of the hepatic bile salt–dependent CEL and the pancreatic enzyme. Although the level of expression of this mRNA in rat liver is variable (8), its molecular identity is now clearly established. CEL cDNA appears to be polymorphic in the rat, as it may also be in humans (18).

Finally, the work reported here now establishes that a single CEL is synthesized and secreted by the liver, the pancreas and the breast, and thus may play diverse roles in mammalian lipid metabolism. Enzyme secreted by the pancreas and that present in milk is most likely involved in the digestion and absorption of dietary, neutral lipid esters (1, 2, 4, 5). The physiologic role of the hepatic enzyme is less clear, although we have speculated that, because it is largely secreted by the liver (11), it may play a role in the metabolism of chylomicron remnants in the space of Disse (19). Very recently, we have detected measurable levels of CEL in human plasma, and shown that the purified porcine pancreatic enzyme can catalyze the hydrolysis of lipid esters in normal and oxidized lipoproteins *in vitro* (20). Thus, it is possible that the largely secreted hepatic CEL functions in the metabolism of a variety of plasma lipoproteins, although more studies are needed to establish this role definitively.

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The sequence data in this paper have been submitted to the EMBL/Gen Bank Data Library under the accession number Z22803.

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