Biochimica et Biophysica Acta, 1174 (1993) 72–74
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BBAEXP 90523

Short Sequence-Paper

Cloning and nucleotide sequence of a novel, male-predominant carboxylesterase in mouse liver

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(Received 22 March 1993)

Key words: Carboxylesterase; Nucleotide sequence; cDNA sequence; Es-male; (Mouse liver)

As a family of serine-dependent enzymes, the carboxylesterases (EC 3.1.1.1) demonstrate a broad substrate specificity. Mouse carboxylesterases comprise at least 20 genetically distinct loci. We cloned a full-length cDNA for a novel mouse carboxylesterase, Es-male which was expressed predominantly in male livers. This carboxylesterase consisted of 554 amino acid residues, and exhibited 43% and 42% similarities to the known mouse esterases Es-22 and pEs-N, respectively. Es-male contained a C-terminal ER-retention signal PEEL, indicating that it may be a microsomal carboxylesterase.

We used λZAPII vector to construct a subtraction cDNA library as described by Klickstein [1] and Goldman and Lafuze [2]. Briefly, double-stranded cDNAs were synthesized from liver mRNAs of [Balb/cJ × DBA/2J] F1 males, ligated to *Eco*RI adaptors. The cDNAs were subtracted by a 50-fold excess of the Balb/cJ cDNAs digested by *Alu*I and *Rsa*I, then ligated to *Eco*RI site of λZAPII vectors. We prepared the F1-enriched, single-stranded cDNAs (F1 probe) using a Subtractor Kit (Invitrogen, San Diego, CA). The subtracted cDNA library was double-screened by F1 and Balb/cJ probes (both single-stranded cDNAs); the clones hybridizing more strongly with the F1 probe were selected. We used mouse albumin and γ-actin cDNAs to judge relative degrees of hybridization. As a result, we obtained 11 different clones which hybridized strongly to the F1 probe but weakly to the Balb/cJ probe.

Clone p1016, one of the 11 cDNA clones, contained a 900 bp insert. We screened the F1 library using the 900 bp insert as probe, and obtained p1016-13 for further characterization. We sequenced both strands of 2036 bp insert of p1016-13: the insert cDNA was digested by *Sau*3AI, *Rsa*I, *Alu*I, *Eco*RI or *Pst*I, ligated to M13 vectors, then sequenced using Sequenase (USB, Cleveland, OH). Fig. 1 shows the nucleotide and deduced amino acid sequences. The nucleotide sequence comprised of 48 bp 5′-noncoding, 1662 bp coding, and 326 bp 3′-noncoding regions. The cDNA-encoded protein consisted of 554 amino acids, and exhibited 43.3% and 41.8% identities to the mouse carboxylesterases Es-22 and pEs-N [3,4], respectively. The closest known carboxylesterase is rabbit form 2 [5]sharing the 44.5% identity. This sequence identity is relatively low when compared with a minimum 60% identity among the 11 rodent, rabbit, pig, and human carboxylesterases already published [3–15]. The p1016-13, however, conserved very well the characteristic sequences for the carboxylesterase family, which included the active-site regions and residues (Asp-109, Ser-214 and His-443) (Fig. 1). Four cysteines (at positions 83, 110, 267 and 278), which may be involved in the specific disulphide bonds, were also conserved. We, therefore, conclude that the p1016-13-enconded protein is a novel carboxylesterase. Proteins which retained in the endoplasmic reticulum (ER) lumen often contain a retention signal at their C-termini [8,16]. This new carboxylesterase also contained a C-terminal ER-retention signal (PEEL). In addition, the enzyme has an N-terminal hybrophobic sequence which may direct its transport into microsomal lumen. The presence of N-terminal and C-terminal signals indicate that the carboxylesterase is a microsome-luminal enzyme. We name the newly-discovered carboxylesterase Es-male because our research indicated that it is expressed predominatly in male livers.

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We determined this male-predominant expression by performing Northern hybridization using ^{32}P-labeled p1016-13 as probe, and examined the mRNA levels of Es-male in the livers of sham-operated, hypophysectomized, and hypophysectomized and growth hormone-treated male and female F1 mice (Fig. 2). The mRNA was expressed at much higher levels in male than female livers. Moreover, the high-level expression in the males was regulated by growth hormone, while the low-level expression in the females was constitutive. The degree of sex-dependency could be underestimated, however, because it is not known how many

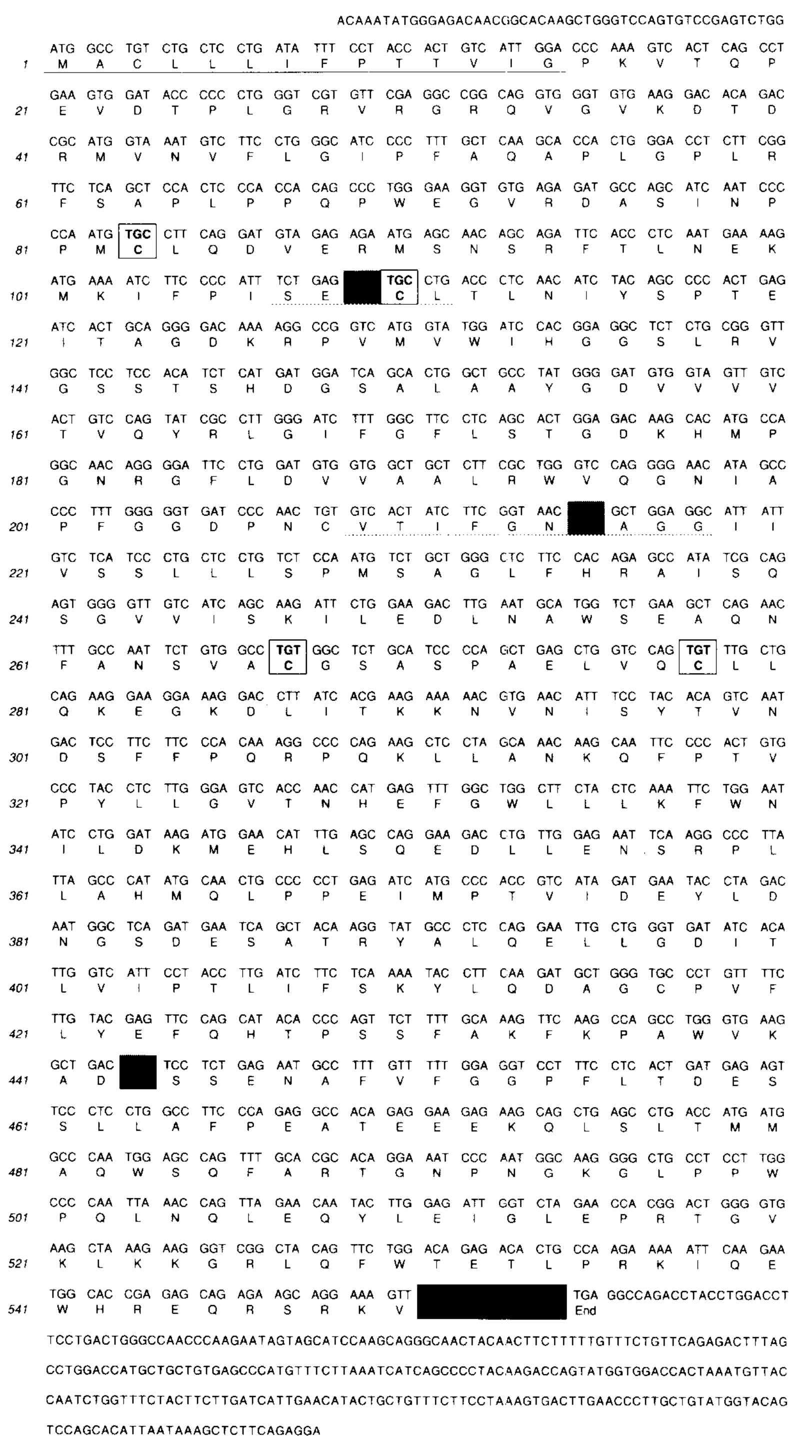

Fig. 1. Nucelotide and deduced amino acid sequences of carboxylesterase Es-male. Three active-site residues are shadowed, while the conserved sequences including the active-site residues are underlined by dotts. Four conserved cysteines are boxed. The N-terminal signal sequence is indicated by solid-underline, whereas the C-terminal retention signal is shaded and boxed. A putative polyA signal is indicated by dashed-underline.

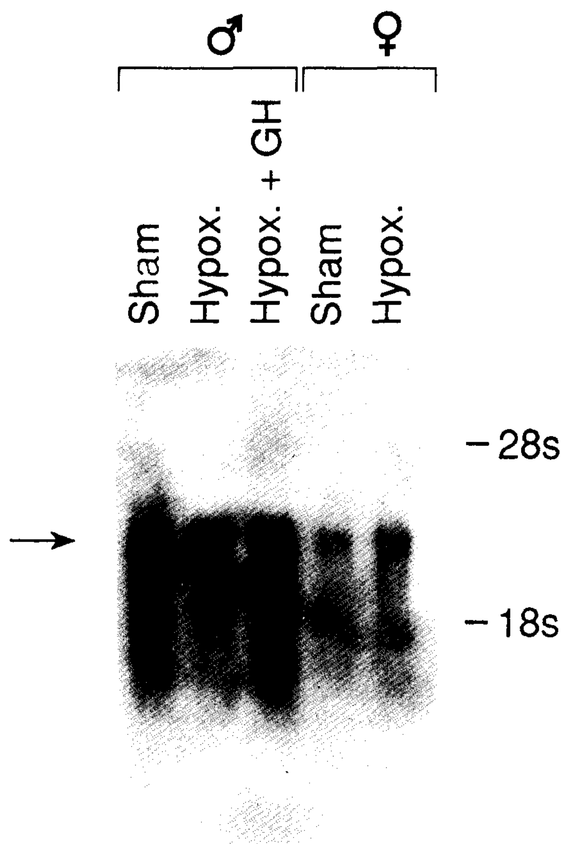

Fig. 2. Male-predominant, GH-dependent expression of Es-male mRNA. Liver RNAs were prepared, enriched for poly(A)-containing RNAs using Oligo-dT cellulose column, electrophoresed on a denatured agarose gel, transferred to Nytran paper, and hybridized by ^{32}P-labeled p1016-13. Wherase the arrow indicates Es-male mRNA, migrations of ribosomal RNAs are indicated by 28 S and 18 S. Sham and Hypox. denote the sham-orerated and hypophyesctomyzed mice. GH shows that mouse was treated by 50 μg of bovine growth hormone (obtained from National Hormone and Pituitary Program) every 12 h for 5 consecutive days.

carboxylesterases are expressed in mouse livers, and to what degree their mRNAs are cross-hybridized. Nevertheless, this regulation-mode in male livers is reminscent of that found in the male-specific steroid 16α-hydroxylase $P450_{16\alpha}$ (2D9) [17]. Mouse Es-1 is known to be a female-predominant plasma carboxylesterase whose mRNA is developmentally increased in female livers [18]. Kadner et al suggest that Es-1 may regulate the estrogen levels, since it hydorlyzes various esters including fatty acid esters of estradiol, and because it is absent in young mice with low estrogen levels [18]. The substrate specificity of Es-male needs to be defined in future in order to speculate a role of this enzyme in male livers.

References

1 Klickstein, L.B. (1988) in Current protocols in molecular biology, Suppl. 4 (Ausybel. F.M., Brent, R., Kingston, R.E., Moore, D.D., Siedman, J.G., Smith, J.A. and Struhl, eds.), pp. 5.8.6–5.8.13. Green Publishing Associated and Wiley Interscience, New York.
2 Goldman, J. and Lafuze, J.E. (1991) Clin. Biotech. 3, 89–93.
3 Ovnic, M., Tepperman, K., Medda, S., Elliott, R.W., Stephenson, D.A., Grant, S.G. and Ganschow, R.E. (1991) Genomics 9, 344–354.
4 Ovnic, M., Swank, R.T., Fletcher, C., Zhen, L., Novak, E.K., Baumann, H., Heintz, N. and Ganschow, R.E. (1991) Genomics 11, 956–967.
5 Ozols, J. (1989) J. Biol. Chem. 264, 12533–12545.
6 Riddles, P.W., Richards, L.J., Bowles, M.R. and Pond, S.M. (1991) Gene 108, 289–292.
7 Matsushima, M., Inoue, H., Ichinose, M., Tsukada, S., Miki, K., Kurokawa, K., Takahashi, T. and Takahasi, K. (1991) FEBS Lett. 193, 37–41.
8 Medda, S. and Proia, R.L. (1992) Eur. J. Biochem. 206, 801–806.
9 Robbi, M., Beaufay, H. and Octavae, J-N. (1990) Biochem. J. 269, 451–458.
10 Munger, J., Shi, G-P., Mark, E.A., Chin, D.C., Gerard, C. and Chapman, H.A. (1991) J. Biol. Chem. 266, 18832–18838.
11 Kroza, G. and Ozols, J. (1988) J. Biol. Chem. 263, 3486–3495.
12 Long, R.M., Satoh, H., Martin, B.M., Kimura, S., Gonzalez, F.J. and Pohl, L.R. (1988) Biochem. Biophys. Res. Commun. 156, 866–873.
13 Zschunke, F., Salmassi, A., Kreipe, H., Buck, F., Pawaresch, M.R. and Radzup, H.J. (1991) Blood 78, 506–512.
14 Takagi, Y., Morohashi, K., Kawabata, S., Go, M. and Omura, T. (1989) J. Biochem. 104, 801–806.
15 Long, R.M., Calabrese, M.R., Martin, B.M. and Pohl, L.R. (1991) Life Sci. 48, 43–49.
16 Pelham, H.R.B. (1990). Trend. Biochem. Sci. 15, 483–486.
17 Noshiro, M. and Negishi, M. (1988) J. Biol. Chem. 261, 15923–15927.
18 Kadner, S.S., Katz, J. and Finlay, T.H. (1992) Arch. Biochem. Biophys. 296, 435–441.