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Short sequence-paper

Cloning and sequencing of the bovine cDNA encoding the mitochondrial tricarboxylate carrier protein [1]

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Abstract

The tricarboxylate or citrate transporter protein (CTP) catalyzes the transport of citrate across the inner mitochondrial membrane by an exchange for malate or some other anionic metabolite. Using primers based on the rat liver cDNA sequence, overlapping cDNA clones encoding the bovine CTP were isolated from bovine liver poly(A^+) cDNA. The entire bovine cDNA is 1151 bp in length with 5′ and 3′ untranslated regions of 7 and 204 bp, respectively. The open reading frame encodes the mature protein consisting of 298 amino acids, preceded by a presequence of 13 amino acids. The amino acid sequence of the mature bovine CTP is 95.6, 94.9, 32.2% identical to that of the citrate carrier from man, rat and yeast, respectively.

Keywords: Tricarboxylate carrier; Citrate carrier; cDNA; Sequence; Mitochondrion

The mitochondrial carriers are a family of proteins which catalyze the transport of important metabolites across the inner mitochondrial membrane (for review, see Refs. [1–4]). These proteins are characterized by a tripartite structure, made up of related sequences about 100 amino acids in length, by the presence of two hydrophobic regions in each of the three repeats and by the three-fold repetition of the sequence motif P-X-D/E-(20–30 amino acids)-D/E-G-(4 amino acids)-aromatic amino acid -K/R-G. One of these transporters is the tricarboxylate or citrate transporter protein (CTP). This carrier, which is present in liver but virtually absent in heart and brain [5,6], catalyzes the efflux of citrate plus a proton from the mitochondrial matrix in an electroneutral exchange for another tricarboxylate-H^+, a dicarboxylate, or phosphoenolpyruvate [7,8]. It therefore plays an important role in fatty acid and sterol syntheses, gluconeogenesis and the transfer of reducing equivalents across the membrane. The CTP has been purified from rat liver mitochondria [9,10], cloned in rat liver [11] and expressed in *Escherichia coli* [12]. The cDNA sequences of the CTP of man [13] and of yeast [14] are also known. These sequences display about 36% identity with a putative CTP from *Caenorhabditis elegans* [15]. Recently the human CTP gene has been sequenced completely [16]. This gene is located within the DiGeorge syndrome critical region of chromosome 22q11 and is hemizygous in the vast majority of DiGeorge syndrome and velo-cardiofacial syndrome patients [13]. Furthermore, it has been indicated that the function of the mitochondrial citrate carrier is altered in type 1 diabetes [17], starvation [18] and cancer [19]. Here we report the nucleotide sequence (and the deduced amino acid sequence) of the bovine CTP determined from overlapping cDNA clones generated by polymerase chain reactions.

Single-stranded cDNA was prepared from bovine liver mRNA with random primers or an oligo(dT) primer and reverse transcriptase (Boehringer) by incubating for 60 min at 55°C. This cDNA was amplified using synthetic oligonucleotide primers based on the rat liver citrate carrier cDNA [11]. PCR amplifications were performed, under conditions described previously [20,21], with deoxynucleosides triphosphate and Taq DNA polymerase (Perkin Elmer) for 30 cycles (or 60 cycles when necessary): 30 s at 94°C, 1 min at 55°C and 1.5 min at 72°C. At the end, a

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[1] The sequence reported in this paper has been deposited with the EMBL Data Library under Accession no. X97773.

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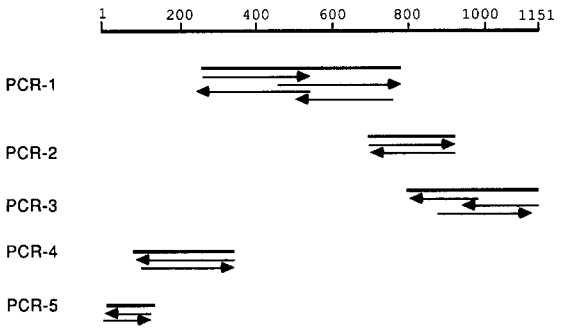

Fig. 1. Generation by polymerase chain reactions and sequence analysis of clones encoding the CTP from bovine liver. PCR-1 to PCR-5 are the cDNA segments generated by PCR. The heavy horizontal lines are proportional to the lengths of these cDNA segments, and the arrows represent the directions and the extents of the determined DNA sequences. The scale is in bases. Forward and reverse primers corresponded to the following nucleotides of the rat cDNA sequence [11]: 259–284 and 755–780 for PCR-1, 730–753 and 907–932 for PCR-2, 850–875 and poly(T) for PCR-3, 64–89 and 301–325 for PCR-4, poly(T) and (as nested reverse primers) 105–125 and 125–145 for PCR-5. In order to analyze the products of PCR-3 and PCR-5, probes corresponding to nucleotides 883–900 and 88–105, respectively, of the rat cDNA were used.

single incubation at 72°C for 7 min was added. In order to extend the sequence towards the 5′-end, the single-stranded cDNA was primed with random hexanucleotides. The products were tailed at their 5′-ends with A residues in the presence of terminal deoxynucleotide transferase (Boehringer). The A-tailed cDNA was used for reaction PCR-5. The PCR products were run on agarose gel, Southern-blotted and hybridized with radioactively labelled synthetic oligonucleotides. Fragments that hybridized with the probes were recovered from the gel by means of the Gene Clean procedure (Bio 101). The products were cloned into M13mp18 and M13mp19 vectors and sequenced in both directions by the modified dideoxy chain termination method [22] with the aid of the modified T7 DNA polymerase (Sequenase, U.S. Biochemicals). In order to avoid sequence errors introduced by the PCR, at least three independent clones were sequenced.

Five overlapping DNA fragments of different lengths containing the sequence of the bovine CTP were obtained (Fig. 1). Fig. 2 shows the nucleotide sequence and the deduced amino acid sequence of the bovine liver cDNA encoding the citrate carrier protein. The sequence of 1151 nucleotides reveals a 936-nucleotide open reading frame extending from the start codon ATG to the termination codon TAA. This cDNA sequence encodes a 311-amino acid protein with an estimated molecular weight of 33925 Da. Comparison of the deduced amino acid sequence with that of the CTP of rat [11], man [13] and yeast [14] and with that of the putative CTP of *C. elegans* is shown in Fig. 3. The coding region of the bovine CTP conserves 87.7% homology with rat, 89.4% with human and 24.5%

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        M  P  A  P  R  A  P  R  A  L  P  S  A  S  P  G  S  G  K  A  K  L  T  H  P  G  K  A
CGCCGCCATGCCTGCGCCTCGCGCACCCCGCGCTCTGCCCTCTGCCTCGCCTGGGTCTGGGAAAGCCAAACTGACACACCCAGGGAAGGC
      10        20        30        40        50        60        70        80        90
  I  L  A  G  G  L  A  G  G  I  E  I  C  I  T  F  P  T  E  Y  V  K  T  Q  L  Q  L  D  E  R
GATCCTAGCAGGCGGCCTGGCTGGCGGCATCGAAATCTGCATCACTTTCCCCACCGAGTACGTGAAGACGCAGCTGCAGCTGGACGAGCG
     100       110       120       130       140       150       160       170       180
  S  H  P  P  R  Y  R  G  I  G  D  C  V  R  Q  T  V  R  S  H  G  L  L  G  L  Y  R  G  L  S
CTCGCACCCGCCGCGCTACCGGGGCATCGGGGACTGCGTGCGGCAGACGGTTCGCAGCCATGGGCTCCTGGGCCTGTACCGCGGCCTTAG
     190       200       210       220       230       240       250       260       270
  S  L  L  Y  G  S  I  P  K  A  A  V  R  F  G  T  F  E  F  L  S  N  H  M  R  D  A  Q  G  R
CTCCCTGCTCTACGGCTCCATCCCCAAGGCGGCCGTCAGGTTCGGGACGTTCGAATTTCTCAGCAACCACATGCGGGACGCCCAGGGACG
     280       290       300       310       320       330       340       350       360
  L  D  S  T  R  G  L  L  C  G  L  G  A  G  V  P  E  A  V  V  V  V  C  P  M  E  T  I  K  V
GCTGGACAGCACGCGCGGGCTGCTGTGCGGCCTGGGCGCCGGAGTGCCAGAGGCCGTGGTGGTCGTGTGCCCCATGGAGACCATCAAGGT
     370       380       390       400       410       420       430       440       450
  K  F  I  H  D  Q  T  S  A  S  P  K  Y  R  G  F  F  H  G  V  R  E  I  V  R  E  Q  G  L  K
GAAATTTATCCATGACCAGACCTCTGCCAGCCCCAAATACCGAGGATTCTTCCACGGGGTCAGGGAGATCGTGCGGGAACAGGGGCTGAA
     460       470       480       490       500       510       520       530       540
  G  T  Y  Q  G  L  T  A  T  V  L  K  Q  G  S  N  Q  G  I  R  F  F  V  M  T  S  L  R  N  W
GGGGACGTATCAAGGCCTCACAGCCACCGTGCTGAAGCAGGGATCCAATCAGGGCATCCGCTTCTTCGTCATGACTTCCCTGCGCAACTG
     550       560       570       580       590       600       610       620       630
  Y  R  G  D  N  P  N  K  P  M  N  P  L  I  T  G  V  F  G  A  I  A  G  A  A  S  V  F  G  N
GTACCGAGGAGACAACCCCAACAAACCCATGAACCCACTGATCACTGGTGTGTTCGGAGCCATCGCAGGCGCAGCCAGTGTCTTCGGGAA
     640       650       660       670       680       690       700       710       720
  T  P  L  D  V  I  K  T  R  M  Q  G  L  E  A  H  K  Y  R  N  T  L  D  C  G  L  Q  I  L  R
CACTCCACTGGACGTGATCAAGACCCGGATGCAGGGACTGGAAGCACACAAGTACCGGAACACGTTGGACTGCGGCCTGCAGATCCTGAG
     730       740       750       760       770       780       790       800       810
  N  E  G  L  K  A  F  Y  K  G  T  V  P  R  L  G  R  V  C  L  D  V  A  I  V  F  I  I  Y  D
GAACGAGGGACTCAAGGCGTTCTACAAGGGCACTGTCCCGCGCCTCGGCCGAGTCTGCCTGGACGTGGCCATCGTGTTCATCATCTACGA
     820       830       840       850       860       870       880       890       900
  E  V  V  K  L  L  N  K  V  W  K  A  D  *
CGAGGTGGTGAAGCTTCTCAACAAAGTGTGGAAGGCGGACTGAGCCCTGGGGTCTGCGCGCGGGCCGTCCCCAGCCGCCCTCTTACAATT
     910       920       930       940       950       960       970       980       990

CCAGCGCAGTCGTCGGAAAAGGCCCTTCCCTGTCCCGCGTGCTCCGTGGCCTGGGTGTGCCGTGCCGGGTCCTCACGTCCTCTGTGGTCT
    1000      1010      1020      1030      1040      1050      1060      1070      1080

TGTATCCACCACCACAATGTCTGTGTCCACGTGAGACTGTGTGTCCTCTCCTGTGAATCTTGCCGCTTGTC
    1090      1100      1110      1120      1130      1140      1150
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Fig. 2. Nucleotide and deduced amino acid sequence of the cDNA encoding the precursor of the bovine liver CTP.

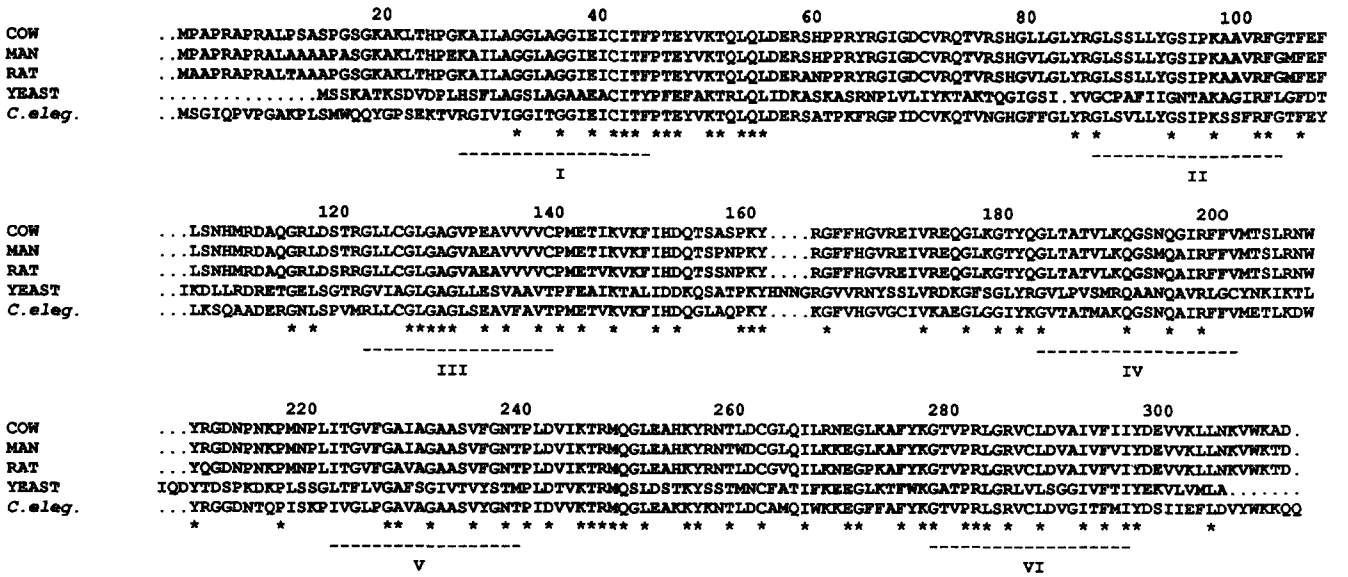

Fig. 3. Alignment of mitochondrial citrate carrier sequences. The bovine precursor CTP sequence was compared with the CTP sequence from rat [11], man [13,16] and yeast [14] and with a putative CTP sequence from *C. elegans* [15]. The numbers above the sequences are related to the bovine sequence. The asterisks indicate identical residues in all five sequences. The dotted lines beneath the sequences refer to the locations of putative membrane-spanning α-helices (I–VI) within the CTP sequence.

with yeast in the nucleotide sequence, and 93.8% with rat, 94.5% with human and 32.2% with yeast in the deduced amino acid sequence. Comparing the five species, 83 amino acids are totally conserved and 106 amino acids are substituted in a highly conservative manner. The identical amino acids include those that form the characteristic sequence motif of the mitochondrial carrier family. Like the CTP of rat [11] and man [13], but not that of yeast [14], the bovine CTP has a processed mitochondrial targeting sequence before the N-terminus of the mature protein. Assuming that the bovine CTP precursor is cleaved at the same position as that from rat [11], the mature bovine CTP consists of 298 amino acids and is preceded by a presequence of 13 amino acids. The bovine and human prosequences differ in two amino acids, and the bovine and rat ones in three amino acids. The two arginine residues that confer the positive charge characteristic of the mitochondrial targeting sequence are conserved. The mature bovine CTP sequence differs in 17 amino acids from that of man and in 19 amino acids from that of rat. Out of these differences 4 between cow and man and 3 between rat and man are non-conservative. The CTP from cow is much less homologous with that from yeast, since they differ in 202 amino acids out of 298, 57 of which are substituted in a highly conservative manner. Comparison of the sequence of the bovine CTP with itself reveals that it has a tripartite structure like the other mitochondrial transport proteins. Furthermore, the hydropathy plot of the bovine CTP suggests the presence of two membrane-spanning segments for each of the three repeats, as originally proposed for the ADP/ATP carrier [23].

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