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Short Sequence-Paper

cDNA cloning of MCT1, a monocarboxylate transporter from rat skeletal muscle ☆

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Abstract

PCR was used to amplify the coding region of CHO MCT1 cDNA. This was then used to screen a rat skeletal muscle cDNA library which lead to the isolation of a full length cDNA encoding MCT1 from rat. The cDNA derived amino acid sequence shows 94% and 86% identity to CHO and human MCT1, respectively.

Keywords: Monocarboxylate; Transporter; MCT1; Skeletal muscle; (Rat)

The transport of lactate and other monocarboxylic acids such as pyruvate, acetoacetate and β-hydroxybutyrate is of major physiological importance in all mammalian cells. Some tissues, such as erythrocytes, tumour cells and white skeletal muscle which use glycolysis to provide for their ATP requirements, must expel lactic acid from the cell. This is also true of cells which are experiencing anoxia. In other tissues there is a requirement for lactic acid to be transported into cells, either for conversion into glucose by gluconeogenic tissues such as the liver and kidney, or for utilisation as a respiratory fuel by cardiac and red skeletal muscle, (see Ref. [1] for a general review).

The transport of monocarboxylates across the plasma membrane of most mammalian cells utilises a proton-linked co-transporter which has been most extensively characterised in erythrocytes and Ehrlich–Lettré tumour cells. In these tissues the carrier has been shown to be stereoselective for L-over D-lactate, and sensitive to inhibition by derivatives of α-cyanocinnamate, organomercurial thiol reagents, and some stilbene disulfonates [2,3]. There is increasing evidence that other tissues possess additional isoforms of the monocarboxylate carrier, which vary in their substrate and inhibitor specificities. This has been demonstrated most clearly in cardiac myocytes [4]. Subtle differences in transport properties in some other tissues such as liver and skeletal muscle suggest that these may also express additional isoforms [1,5,6].

Recently a cDNA encoding MonoCarboxylate Transporter 1 (MCT1) was cloned from Chinese Hamster Ovary (CHO) cells [7]. The cellular distribution of MCT1 in various Syrian hamster tissues was studied by Northern blotting [8], immunoblotting and immunofluorescence microscopy [7,9]. The largest amounts of transporter were seen in erythrocytes, caecum, heart, eye, lung, epididymis, and testis. Intermediate levels were seen in the brain and kidney, while low levels were detected in skeletal muscle and liver. Interestingly, the immunofluorescence studies demonstrated that only a subset of skeletal muscle fibres were immunopositive. These fibres also stained for succinate dehydrogenase, thus identifying them as mitochondria rich. Since both white skeletal muscle and liver are important sites for monocarboxylate transport, these observations provide further evidence for the existence of different transporter isoforms. It was therefore decided to clone the cDNAs for the skeletal muscle isoforms in order to carry out analysis of structure and function. These molecular studies were undertaken using rat, since this was the experimental system used in most of our previous kinetic analyses. The rat is also the animal used most extensively in metabolic studies.

A cDNA probe was generated against the coding region of CHO MCT1. Total RNA was prepared from CHO cells

☆ The sequence data reported in this paper have been deposited in the EMBL Data Bank under the accession number X86216.

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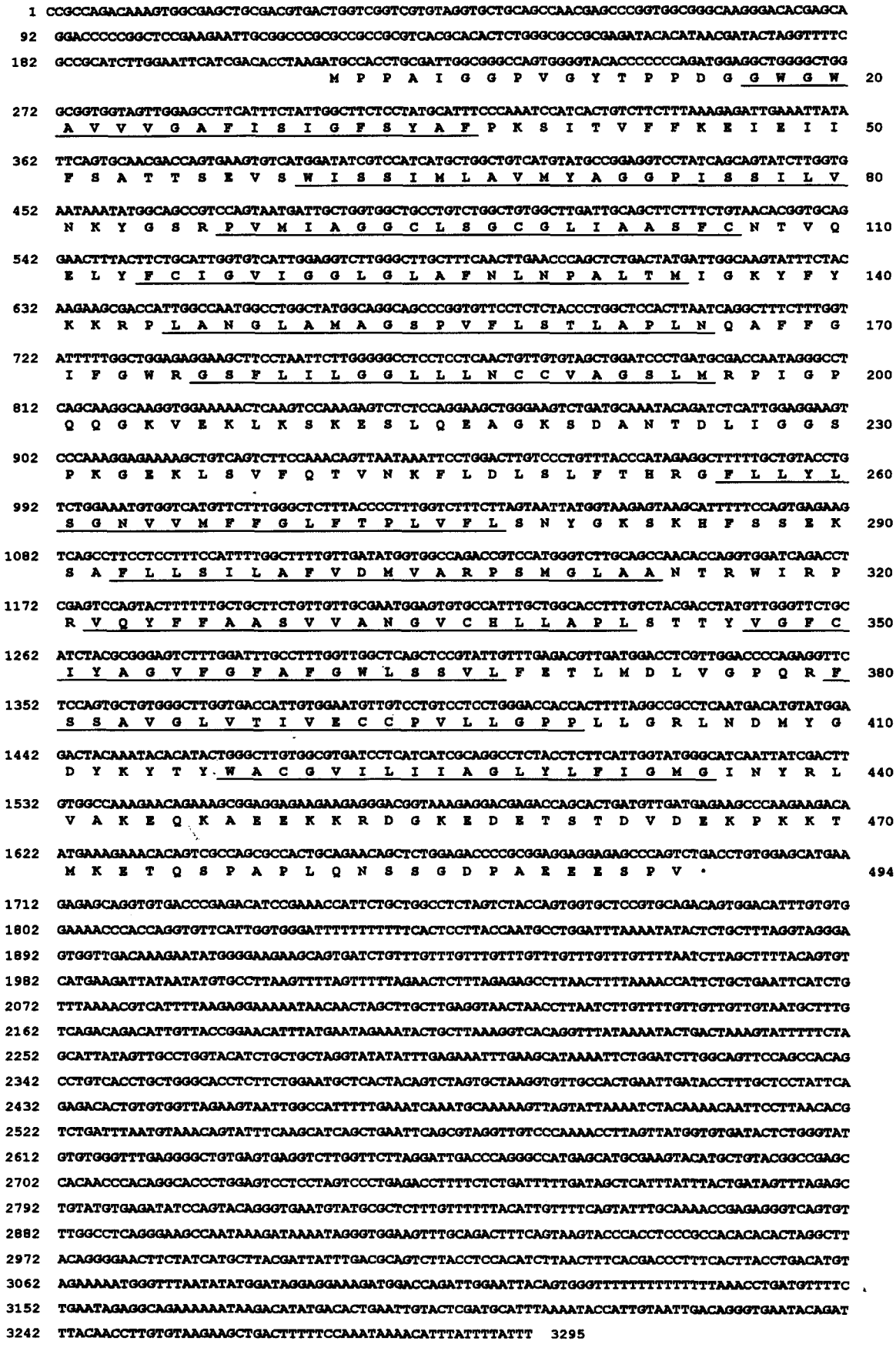
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632 AAGAAGCGACCATTGGCCAATGGCCTGGCTATGGCAGGCAGCCCGGTGTTCCTCTCTACCCTGGCTCCACTTAATCAGGCTTTCTTTGGT
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722 ATTTTTGGCTGGAGAGGAAGCTTCCTAATTCTTGGGGGCCTCCTCCTCAACTGTTGTGTAGCTGGATCCCTGATGCGACCAATAGGGCCT
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1442 GACTACAAATACACATACTGGGCTTGTGGCGTGATCCTCATCATCGCAGGCCTCTACCTCTTCATTGGTATGGGCATCAATTATCGACTT
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M K E T Q S P A P L Q N S S G D P A E E E S P V • 494
1712 GAGAGCAGGTGTGACCCGAGACATCCGAAACCATTCTGCTGGCCTCTAGTCTACCAGTGGTGCTCCGTGCAGACAGTGGACATTTGTGTG
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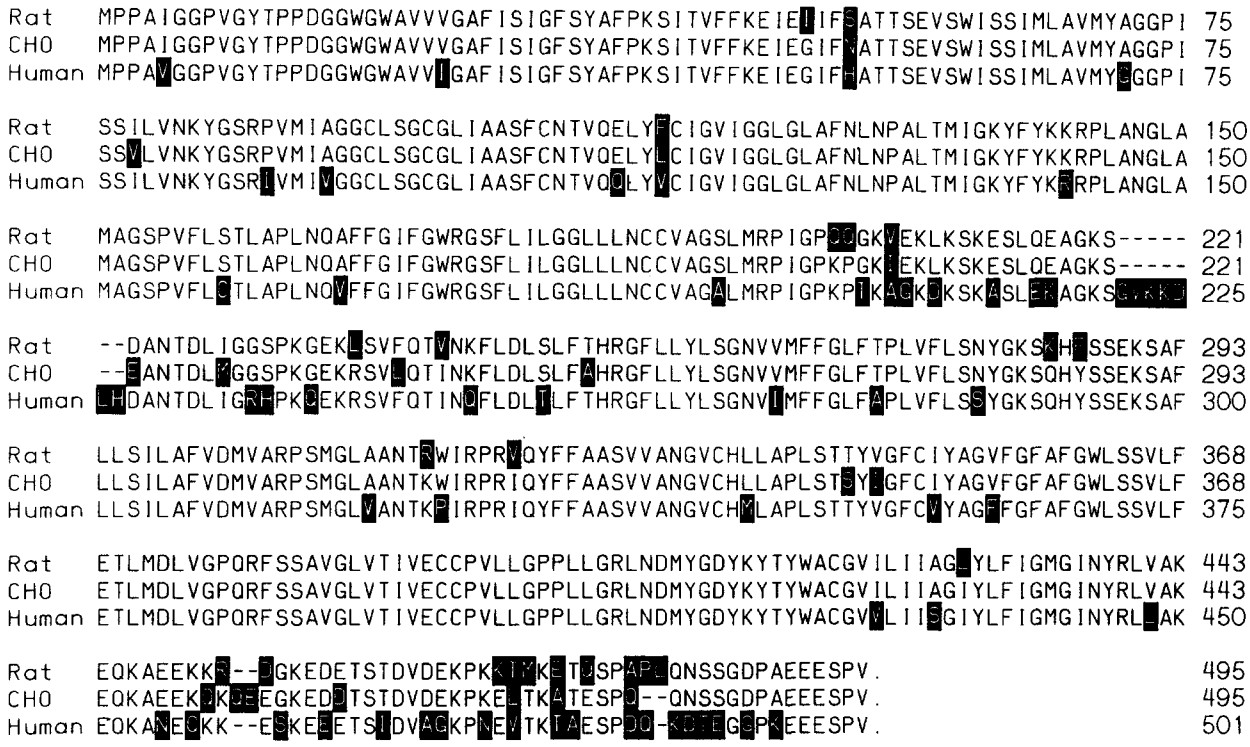

Fig. 2. Alignment of the protein sequences of rat, CHO and human MCT1. Alignments were performed using Megalign software (DNAStar) with the Clustal algorithm (gap penalty = 10, gap length penalty = 10). Residues which are not identical in all three sequences are highlighted.

and cDNA was synthesised using AMV reverse transcriptase with oligo(dT) priming. PCR was performed for 35 cycles using standard conditions with exact match primers flanking the coding region of the CHO sequence. A single band of the predicted size (1500 bp) was obtained. The purified PCR product was labelled with [α-^{32}P]dCTP by random priming (Amersham Multiprime Kit). The probe was then purified by gel filtration (Pharmacia Nick Column) and used to screen a rat skeletal muscle Uni-ZAP XR library (Stratagene, La Jolla, CA, USA). Putative positive clones obtained from screening approximately $1 \cdot 10^6$ recombinant phage were then subjected to two further rounds of screening until plaque purity was achieved. The pBluescript phagemids were then excised using the ExAssist helper phage as described by Stratagene. Initial characterisation of the inserts by multiple restriction digests showed that several of the clones were identical, both in size and restriction pattern and thus were likely to be sibling clones. Initial sequencing of the 3′ and 5′ ends of one insert showed it to be very similar to the corresponding non-coding regions of CHO MCT1. This 3.3 kb insert was then sequenced on both strands using a custom primer walking strategy on a Du Pont Genesis 2000 automated sequencer.

The complete cDNA and deduced amino acid sequence obtained are presented in Fig. 1. The sequence contains an open reading frame encoding 494 amino acids. This predicts a polypeptide of 53 kDa having 12 putative transmembrane spanning regions. In MCT1 purified from rabbit erythrocytes, the N-terminal sequence of the mature protein corresponds to this predicted ORF, except that the initiator methionine is removed, and there is an isoleucine to valine substitution at position five [10].

The 3295 bp long cDNA contains 211 bp of 5′ non-coding sequence and 1597 bp of 3′ non-coding sequence. During the course of this work, the sequence of human MCT1 was also published [11]. Overall the rat nucleotide sequence has 89% identity with CHO MCT1, and 83% identity with human MCT1.

The derived amino acid sequence of rat MCT1 has 94% and 86% identity with CHO and human MCT1, respectively. Fig. 2 shows an alignment of the predicted amino acid sequences. As expected the sequences have the highest degree of homology within the predicted membrane-spanning regions. Unlike CHO MCT1, the rat and human sequences do not possess a potential site for N-linked glycosylation (Asn-X-Ser/Thr); Asparagine 52 is replaced by serine in rat and histidine in the human sequence.

Fig. 1. Nucleotide and deduced amino acid sequences of rat MCT1 cDNA. Numbers on the left refer to nucleotides, while those on the right refer to the amino acids. The underlined regions of the protein sequence denote the predicted transmembrane regions, assigned on the basis of hydrophobicity.

However, despite the presence of this consensus motif, recent experimental results show that the CHO MCT1 protein is not glycosylated [12].

The deduced amino acid sequence of rat MCT1 will allow site directed mutations to be generated. These may help to identify those residues responsible for the catalytic mechanism of monocarboxylate transport and also those residues which confer substrate and inhibitor specificity. Mutation of phenylanine 360 to cysteine in transmembrane region ten of CHO MCT1 causes a gain of function allowing transport of mevalonate [8]. This region is thus intimately involved in the transport process. This phenylalanine is present at the same position within transmembrane region 10 in all of the MCT1 sequences isolated so far.

Recently a cDNA encoding a second monocarboxylate transporter isoform, MCT2, was cloned from hamster liver [9]. This cDNA has also been shown to be expressed in oxidative skeletal muscle, but not in white skeletal muscle, as is the case for MCT1. Thus it is likely that another isoform of MCT exists in the glycolytic fast twitch fibres. The rat MCT1 cDNA isolated in this study is likely to derive from red muscle present in the mixed muscle fibres of the hind limb tissue used to generate the library.

Studies are currently under way to isolate an MCT isoform from white skeletal muscle fibres. We are also characterising several other clones from the mixed fibre library which appear to be different from MCT1.

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References

[1] Poole, R.C. and Halestrap, A.P. (1993) Am. J. Physiol. 264, C761–C782.
[2] Poole, R.C. and Halestrap, A.P. (1991) Biochem. J. 275, 307–312.
[3] Carpenter, L. and Halestrap, A.P. (1994) Biochem. J. 304, 751–760.
[4] Wang, X., Levi, A.J. and Halestrap, A.P. (1994) Am. J. Physiol. 267, H1759–H1769.
[5] Edlund, G.L. and Halestrap, A.P. (1988) Biochem. J. 249, 117–126.
[6] Roth, D.A. and Brooks, G.A. (1990) Arch. Biochem. Biophys. 279, 377–385.
[7] Garcia, C.K., Goldstein, J.L., Pathak, R.K., Anderson, R.G.W. and Brown, M.S. (1994) Cell 76, 865–873.
[8] Kim, C.M., Goldstein, J.L. and Brown, M.S. (1992) J. Biol. Chem. 267, 23113–23121
[9] Garcia, C.K., Brown, M.S., Pathak, R.K. and Goldstein, J.L. (1995) J. Biol. Chem. 270, 1843–1849.
[10] Poole, R.C. and Halestrap, A.P. (1994) Biochem. J. 303, 755–759.
[11] Garcia, C.K., Li, X., Luna, J. and Francke, U. (1994) Genomics 23, 500–503.
[12] Carpenter, L., Poole, R.C. and Halestrap, A.P. (1995) Biochem. J., manuscript submitted.