CLONING TWO PepT1 cDNA FRAGMENTS OF COMMON CARP, CYPRINUS CARPIO (ACTINOPTERYGI: CYPRINIFORMES: CYPRINIDAE)

Teresa OSTASZEWSKA 1*, Iwona SZATKOWSKA 2, Tiziano VERRI 3, Konrad DABROWSKI 4, Alessandro ROMANO 3, Amilcare BARCA 3, Magdalena MUSZYŃSKA 2, Andrzej DYBUS 2, Piotr GROCHOWSKI 1, and Maciej KAMASZEWSKI 1

1 Division of Ichthyobiology and Fisheries, Faculty of Animal Science, Warsaw University of Life Sciences, Warsaw, Poland
2 Division of Molecular Cytogenetics, Faculty of Biotechnology and Animal Science, West Pomeranian University of Technology, Szczecin, Poland
3 Laboratory of General Physiology, Department of Biological and Environmental Sciences and Technologies, University of Salento, via Provinciale Lecce-Monteroni, Lecce, Italy
4 School of Environment and Natural Resources, Ohio State University, Columbus, Ohio, USA

Ostaszewska T., Szatkowska I., Verri T., Dabrowski K., Romano A., Barca A., Muszyńska M., Dybus A., Grochowski P., Kamaszewski M. 2009. Cloning two PepT1 cDNA fragments of common carp, Cyprinus carpio (Actinopterygii: Cypriniformes: Cyprinidae). Acta Ichthyol. Piscat. 39 (2): 81–86.

Background. Common carp, Cyprinus carpio, is a model organism within Teleostei. Oligopeptides are a new and promising alternative source of amino acids in animal as well as in human nutrition. In common carp, the membrane protein that transports oligopeptides across the enterocyte membrane is encoded by the gene PepT1 (SLC15A1). The aim of this paper was to sequence the PepT1 (SLC15A1) in common carp.

Materials and Methods. Intestine samples were isolated from six-week old common carp. Total RNA was isolated using a Trizol method. Reverse transcription was used to synthesize cDNA. Two different pairs of primers were designed, according to the zebrafish (Danio rerio) PepT1 sequence, and used for PCR. The amplified DNA was isolated by electrophoresis, cloned (pCRII-TOPO vectors), sequenced, and subjected to in silico analysis.

Results. Two nucleotide fragments of the PepT1 gene were obtained and analyzed using bioinformatic tools. Both fragments showed a high degree of homology with the known PepT1 genes of other teleosts, mammals, and birds. High homology of the PepT1 gene, and similar primary protein structure among the aforementioned taxa probably reflects the conservative function of the PepT1 protein product. Both fragments of the PepT1 gene were deposited in GenBank (FJ556590; FJ529670).

Conclusions. The sequenced fragments of the common carp PepT1 gene will allow evaluation of PepT1 expression in the intestines of fish fed diets containing various forms of protein, which is an issue of importance regarding fish nutrition and its import for aquaculture.

Keywords: Cyprinus carpio, peptide transporter, PepT1, intestine, sequence

INTRODUCTION

In genome sequencing of prokaryotes and eukaryotes two strategies are applied. The first, involves sequencing the entire genome. The second, and less common, approach involves sequencing specific parts of the genome, usually a single gene. However, irrespective of the strategy applied, the selection of organisms for genomic research is a most important task. The most commonly applied criterion regulating such selection, besides that of “model organism” (e.g., mouse, or rat), is consideration of the biological and commercial importance of an organism, a consideration aimed at guaranteeing that the genomic results would be practically applied. This criterion is relevant to common carp (Cyprinus carpio), for which small sections of nuclear and mitochondrial genomes have been sequenced, but for which specifics regarding genome structure, regulation of gene expression, and molecular determination of features relevant to...
commercial interest still need to be elucidated. Therefore, partial sequencing of the common carp PepT1 gene (called also SLC15A1 – SoLute Carrier 15 A1), coding for an oligopeptide membrane transporter protein was assumed meaningful for some reasons: its expression is related to digestive tract differentiation and development, digestion physiology, and nutrient transport. Up to now the PepT1 sequences were described among other fish: zebrafish, *Danio rerio* (see: Verri et al. 2003), icefish, *Chionodraco hamatus* (see: Maffia et al. 2003), and Atlantic cod, *Gadus morhua* (see: Amberg et al. 2008). These issues are of key importance for optimization of stocking material pre-rearing technique development. With the above in mind, we herein report research findings from a study designed to sequence the PepT1 (*SLC15A1*) in common carp.

**MATERIALS AND METHODS**

PCR-based cloning of cDNA fragments encoding for carp PepT1. Common carp intestine was isolated from 6-week-old fish (5 individuals) killed by overdose immersion in the anaesthetic 3-aminobenzoic acid ethyl ester (MS-222 Sigma-Aldrich, Poznań, Poland). Following euthanasia, the entire length of the intestine was removed. Tissue was briefly rinsed in ice-cold saline solution (1.1% NaCl), immediately fixed in RNA later (Ambion, Inc., Austin, TX, US), and stored at –80°C until use.

Total RNA was extracted from the RNAlater-stored intestine using Trizol Reagent (Invitrogen, Carlsbad, CA, US) according to the manufacturer’s instructions. Reverse transcription was performed at 50°C for 60 min using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, US), and stored at –80°C until use.

Two microliters of resulting cDNA product was used to perform PCR with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, US) in the presence of oligo(dT)12–18 primer and US) according to the manufacturer’s instructions. Reverse transcription was performed at 50°C for 60 min using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA, US), and stored at –80°C until use. ORF finder program at NCBI website

PCR amplification was carried out for 35 cycles, with denaturation at 94°C for 2 min, PCR amplification was carried out for 35 cycles, with denaturation at 94°C for 2 min, annealing at 50°C for 60 s, and extension at 72°C for 60 s, and a final extension at 72°C for 7 min. RT-PCR products were separated by electrophoresis on a 1% agarose gel and stained with ethidium bromide. PCR amplification products of the expected size were eluted using the QIAquick Gel Extraction kit (Qiagen, Chatsworth, CA, US) and subsequently cloned in the pCRII-TOPO vector (TOPO TA Cloning, Invitrogen Carlsbad, CA, US). The plasmid clones containing the isolated cDNA fragments were sequenced using universal primers.

*In silico analysis.* The identity of cDNA fragments were confirmed by BLAST comparison with the GenBank database on the NCBI server (http://www.ncbi.nlm.nih.gov/BLAST). Partial carp PepT1 amino acid sequences were deduced using the ORF finder program at NCBI website (www.ncbi.nlm.nih.gov). Pairwise and multiple sequence alignments were carried out using the ClustalW program (http://www.ebi.ac.uk/clustalw). GenBank accession numbers for sequence comparisons were: human, *Homo sapiens* PepT1 (AAB61693; Liang et al. 1995), rabbit, *Oryctolagus cuniculus* PepT1 (AAA17721; Fei et al. 1994), rat, *Rattus norvegicus* PepT1 (BAA09318; Miyamoto et al. 1996), mouse, *Mus musculus* PepT1 (AAF81666; Fei et al. 2000), sheep, *Ovis aries* PepT1 (AAK14788; Pan et al. 2001), pig, *Sus scrofa* PepT1 (AAO43094; Klang et al. 2005), dog, *Canis lupus familiaris* PepT1 (AAL67837; Madin-Darby canine kidney cell), chicken, *Gallus gallus* PepT1 (AAK39954; Chen et al. 2002), turkey, *Meleagris gallopavo* PepT1 (AAO16604; Van et al. 2005), zebrafish, *Danio rerio* PepT1 (AAQ65244; Verri et al. 2003), icefish, *Chionodraco hamatus* PepT1 (AAO39705; Maffia et al. 2003), and Atlantic cod, *Gadus morhua* PepT1 (AY921634; Rønneset et al. 2007).

| Table 1 |
| --- |
| Nucleotide sequence of the first fragment of common carp, *Cyprinus carpio*, PepT1 gene divided into codons, and inferred primary protein structure |

Yellow—conservative sequences among fish species of PepT1 gene; Blue—conservative sequences among vertebrate species of PepT1 gene.
Two PepT1 cDNA fragments from common carp were cloned, sequenced and translated into their potential amino acid sequence (Tables 1, 2).

Equally high similarity at the DNA level is also observed for mammals and birds; however, only two species of the latter were compared. Inferred amino acid sequences from this fragment spanned from transmembrane domain 3 (TM 3) to TM 4 and exhibited very high (86%) similarity to the zebrafish PepT1 amino acid sequence (Tables 2, 4). The same refers to the taxa mentioned above.

Similarly, the second cDNA fragment exhibited 87.5% similarity to the zfPepT1 gene fragment and over 64% with respect to the three teleosts for which sequences were made available (Table 3).

RESULTS
Two PepT1 cDNA fragments from common carp were cloned, sequenced and translated into their potential amino acid sequence (Tables 1, 2).

Equally high similarity at the DNA level is also observed for mammals and birds; however, only two species of the latter were compared. Inferred amino acid sequences from this fragment spanned from transmembrane domain 3 (TM 3) to TM 4 and exhibited very high (86%) similarity to the zebrafish PepT1 amino acid sequence (Tables 2, 4). The same refers to the taxa mentioned above.
### Table 4
Comparison of amino acid sequence of PepT1 protein for various vertebrates available from the GenBank database—fragment 1

| Species    | Amino Acid Sequence                      | Length |
|------------|------------------------------------------|--------|
| Human      | IVYTIQAVTVSINLDTQDNHDDTFSFLPVTVLVLSLIG | 143    |
| Rabbit     | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 143    |
| Rat        | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 143    |
| Mouse      | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 143    |
| Sheep      | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 143    |
| Pig        | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 143    |
| Dog        | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 143    |
| Chicken    | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 149    |
| Turkey     | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 149    |
| Zebrafish  | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 150    |
| Icefish    | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 69     |
| Atlantic Cod | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 150    |
| Carp       | IVYTIQAVTVSISNEISDNHDSTFSFLPVTVLVLSLIG | 49     |

Yellow—conservative sequences among fish species of PepT1 gene; Blue—conservative sequences among vertebrate species of PepT1 gene.

### Table 5
Comparison of amino acid sequence of PepT1 protein for various vertebrates available from the GenBank database—fragment 2

| Species    | Amino Acid Sequence                      | Length |
|------------|------------------------------------------|--------|
| Human      | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Rabbit     | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Rat        | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Mouse      | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Sheep      | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Pig        | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Dog        | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 208    |
| Chicken    | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 214    |
| Turkey     | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 214    |
| Zebrafish  | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 215    |
| Icefish    | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 134    |
| A. Cod     | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 215    |
| Carp       | GQKQKRFKFFSLYLAHGSLBSTDIIIFPLVQGCWYSQH | 52     |

Human: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Rabbit: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Rat: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Mouse: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Sheep: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Pig: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Dog: 209 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 263
Chicken: 215 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 269
Turkey: 215 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 269
Zebrafish: 216 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 270
Icefish: 135 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 189
A. Cod: 216 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 270
Carp: 53 WAVALVFVLSGTHKKFQOGINGKAKACIGAIKGNFRESHSKAFKPREHVLD 107

Human: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Rabbit: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Rat: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Mouse: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Sheep: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Pig: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Dog: 264 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 318
Chicken: 270 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 324
Turkey: 270 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 324
Zebrafish: 271 WAKEYDERLSQOKRKTWFKFLFPIPFALMFDFGMRDGTATTESGKIGL 325
It is noteworthy that considerable conservatism at the level of primary protein structure was observed mainly for transmembrane domain regions (Table 6), particularly in TM 5, while in connecting loops homology was much lower.

**DISCUSSION**

Peptide transporters (PEPT) are membrane proteins responsible for selective transport of small peptides across the intestine enterocyte membranes (Chen et al. 2005). Among them, PepT1 is present in the small intestine villi and is of key importance for absorption of protein hydrolysis products, particularly dipeptides and tripeptides. The PepT1 protein in higher vertebrates shows similar length to each other, usually about 700 amino acid residues (708 in human, 707 in rat, and 709 in mouse,) while in lower vertebrates it tends to be longer. The difference relates rather to the length of loops connecting transmembrane domains than the length of the domains themselves (Liang et al. 1995, Miyamoto 1996, Fei et al. 2000).

Although no crystallographic structure of PepT1 protein is known, a probable model of PepT1 protein was created (Abramson et al. 2003, Huang et al. 2003) using appropriate software, and crystallography of similar transporter proteins of *E. coli* LacY (crystallized bound to the substrate), and GlpT (crystallized without a substrate). PepT1 shows an α-helix structure, and consists of 12 functional transmembrane domains, nonlinearly distributed within the cell membrane. It seems that a hydrophilous cation (H⁺) transmembrane channel exists, through which dipeptides, tripeptides and free amino acids are transported (Meredith and Price 2006). Among the 12 domains, seven are directly involved in channel structure (domains 1, 3, 5, 7, 8, 9, and 10). In the present study, the *in silico* analysis revealed that domains 5, 6, and 7 were the most conservative, while connecting regions (loops) (except for the one connecting domains 7 and 8) were more variable. Particularly domain TM 5, which is responsible for regulation of the rate of substrate transport through the cation channel, showed high level of conservatism (72%). It is similarly to the domains 6 and 7, which participate in initiation of substrate binding, and regulate the rate of their flow (61% and 63%, respectively). It is worth mentioning that an important role of some amino acid radicals was observed in other vertebrates, such as Y167, R282, or W294, substitution of which considerably disturbed biological activity of PepT1 protein (Bolger et al. 1998).

**Table 6**

| Species                  | TM 5 18 aa | Loop 5/6 28 aa | TM 6 19 aa | Loop 6/7 45 aa | TM 7 18 aa | Loop 7/8 37 aa |
|--------------------------|------------|----------------|------------|----------------|------------|----------------|
| Mammals⁴                 | 100        | 64             | 74         | 71             | 89         | 78.5           |
| Birds⁴                  | 100        | 96.5           | 95         | 98             | 100        | 97             |
| Fishes⁵                 | 100        | 78.5           | 84         | 46.5           | 78         | 86             |
| Zebrafish/carp⁶          | 100        | 89             | 88.5       | 80             | 94.5       | 94.5           |
| All species              | 72         | 46.5           | 63         | 44.5           | 61         | 75.5           |

⁴human, *Homo sapiens*; rabbit, *Oryctolagus cuniculus*; rat, *Rattus norvegicus*; mouse, *Mus musculus*; sheep, *Ovis aries*; pig, *Sus scrofa*; dog, *Canis lupus familiaris*; ⁵chicken, *Gallus gallus*; turkey *Meleagris gallopavo*; ⁶zebrafish, *Danio rerio*; icefish, *Chionodraco hamatus*; Atlantic cod, *Gadus morhua*.
In the fragments of PepT1 protein analyzed in the present study these sites are identical as in other species. Concluding, we can assume that high homology of PepT1 gene at the DNA level, and conservative primary structure of PepT1 protein probably reflect PepT1 conservative function, the pattern of expression, or PepT1 level corresponding to the internal environment conditions. The obtained partial sequence of common carp PepT1 should allow to test this hypothesis in future studies.

ACKNOWLEDGEMENTS

The presently reported study has been financed by the Ministry of Science and Higher Education, Republic of Poland, in the frames of the grant No. 311 030 32/ 2256.

REFERENCES

Abramson J., Smirnova I., Kasho V., Verner G., Kaback H.R., Iwata S. 2005. Structure and mechanism of the lactose permease of Escherichia coli. Science 301 (5633): 610–615. DOI: 10.1126/science.1088196.

Amberg J.J., Myr C., Kamisaka Y., Jordal A.-E.O., Rust M.B., Hardy R.W., Koedijk R., Ronnestad I. 2008. Expression of the oligopeptide transporter, PepT1, in larval Atlantic cod (Gadus morhua). Comparative Biochemistry and Physiology, Part B: Biochemistry and Molecular Biology 150: 177–182. DOI:10.1016/j.cbpb.2008.02.011.

Bolger M.B., Haworth I.S., Yeung A.K., Ann D., von Graffenstein M., Lemieux M.J., Song J., Auer M., Wang D.-N. 2003. Structure and mechanism of the glycerol-3-phosphate transporter from Escherichia coli. Science 301 (5633): 616–620. DOI: 10.1126/science.1087619.

Klang J.E., Burnworth L.A., Pan Y.X., Webb K.E.jr. Wong E.A. 2005. Functional characterization of a cloned pig intestinal peptide transporter (pPepT1). Journal of Animal Science 83: 172–181.

Liang R., Fei Y.-J., Prasad P.D., Ramamoorthy S., Han H., Yang-Feng T.L., Hediger M.A., Ganapathy V., Leibach F.H. 1995. Human intestinal H+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. Journal of Biological Chemistry 270: 6456–6463. DOI: 10.1074/jbc.270.12.6456.

Maffia M., Rizzello A., Acierno R., Verri T., Rollo M., Danieli A., Düring F., Daniel H., Storelli C. 2003. Characterisation of intestinal peptide transporter of the Antarctic haemoglobinless teleost Chionodraco hamatus. Journal of Experimental Biology 206: 705–714. DOI: 10.1242/jeb.00145.

Meredith D., Price R.A. 2006. Molecular modeling of PepT1—towards a structure. Journal of Membrane Biology 213: 79–88. DOI:10.1007/s00232-006-0876-6.

Miyamoto K., Shiraga T., Morita K., Yamamoto H., Haga H., Taketani Y., Tamai I., Sai Y., Tsuji A., Takeda E. 1996. Sequence, tissue distribution and developmental changes in rat intestinal oligopeptide transporter. Biochimica et Biophysica Acta – Gene Structure and Expression 1305: 34–38. DOI: 10.1016/0167-4781(95)00208-1.

Pan Y., Wong E.A., Bloomquist J.R., Webb K.E. jr. 2001. Expression of a cloned ovine gastrointestinal peptide transporter (oPepT1) in Xenopus oocytes induces uptake of oligopeptides in vitro. Journal of Nutrition 131: 1264–1270.

Ronnestad I., Gavaia P.J., Viegas C.S., Verri T., Romano A., Nilsen T.O., Jordal A.E., Kamisaka Y., Cancela M.L. 2007. Oligopeptide transporter PepT1 in Atlantic cod (Gadus morhua L.); cloning, tissue expression and comparative aspects. Journal of Experimental Biology 210: 3883–3896. DOI: 10.1242/jeb.007898.

Van L., Pan Y.X., Bloomquist J.R., Webb K.E.jr., Wong E.A. 2005. Developmental regulation of a turkey intestinal peptide transporter (PepT1). Poultry Science 84: 75–82.

Verri T., Kottra G., Romano A., Tiso N., Peric M., Maffia M., Boll M., Argenton F., Daniel H., Storelli C. 2003. Molecular and functional characterisation of the zebrafish (Danio rerio) PepT1-type peptide transporter. Federation of European Biochemical Societies Letters 549: 115–122. DOI: 10.1016/S0014-5793(03)00759-2.

Received: 6 March 2009
Accepted: 3 June 2009
Published electronically: 10 December 2009