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

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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

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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), oligo(dT)_{12–18} primer and 5 μg total RNA following the manufacturer's instructions. Two microliters of resulting cDNA product was used to perform PCR with Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, US) in the presence of zebrafish-specific primer pairs designed according to the nucleotide sequence of zebrafish PepT1 cDNA (*zfpept1*; GenBank acc. no. AY300011) (Verri et al. 2003). Two different primer pairs were synthesized commercially (Proligo France SAS, Paris, France) and used to amplify and clone two conserved fragments of carp PepT1 cDNA. The primer pair *zfPepT1F1* (5'-TGTATCTGTC-TATAGTGTAC-3', identical to nucleotides 387–406 of zebrafish PepT1 cDNA) – *zfPepT1R1* (5'-CAAATGCT-

-GCCACACAC-3', the reverse complement of nucleotides 556–572 of zebrafish PepT1 cDNA) was expected to amplify a 187 bp fragment and the primer pair *zfPepT1F2* (5'-TCAGTTTGAAGATCACCAGG-3', identical to nucleotides 580–599 of zebrafish PepT1 cDNA) – *zfPepT1R2* (5'-AGTATAGGGTTCAC-GATCTG-3', the reverse complement of nucleotides 1109–1128 of zebrafish PepT1 cDNA) was expected to amplify a 549 bp fragment. After a denaturing step at 94°C for 2 min, PCR amplification was carried out for 35 cycles, with denaturation at 94°C for 30 s, 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; Rønnestad 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

ACC	ATC	GGC	CAG	GTG	ATC	ATG	GCA	ATC	AGT	GCC	ATA	CAT	GAC	ATC	ACA	GAT	ACA	AAC	AGA	GAC	GGT	ACA
T	I	G	Q	V	I	M	A	I	S	A	I	H	D	I	T	D	T	N	R	D	G	T
CCT	GAC	AAC	ATG	ACA	TTC	CAT	ACT	GCC	ATG	TCC	ATG	CTG	GGA	CTC	ATC	CTC	ATT	GCT	TTG	GGA	ACT	GGA
P	D	N	M	T	F	H	T	A	M	S	M	L	G	L	I	L	I	A	L	G	T	G
GGT	ATT	AAA																				
G	I	K																				

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

Table 2

Nucleotide sequence of the second fragment of common carp, *Cyprinus carpio*, PepT1 gene divided into codons, and inferred primary protein structure

AAA	CAA	AGA	AGT	ACT	TTC	TTT	TCC	ATA	TTT	TAT	CTG	TCC	ATT	AAT	GCT	GGC	AGT	CTT	CTC	TCC	ACT	CTC
K	Q	R	S	T	F	F	S	I	F	Y	L	S	I	N	A	G	S	L	L	S	T	L
ATC	ACA	CCC	ATC	CTG	AGA	GCT	CAA	GAG	TGT	GGT	ATC	TAT	TCA	AAG	CAA	AGT	TGT	TTC	CCG	CTG	GCT	TTT
I	T	P	I	L	R	A	Q	E	C	G	I	Y	S	K	Q	S	C	F	P	L	A	F
GGG	GTC	CCT	GCC	GCT	CTC	ATG	GTG	GTT	GCC	CTC	ATT	GTG	TTC	ATC	GCA	GGC	CAC	AAC	ATG	TAC	ATT	ATG
G	V	P	A	A	L	M	V	V	A	L	I	V	F	I	A	G	H	N	M	V	I	M
GAA	TCC	CCC	AAA	GGC	AAC	ATC	CTG	CTG	CAA	GTC	ATG	AAG	TGC	ATT	GGA	TTT	GCA	ATT	AGG	AAC	CGT	TTC
E	S	P	K	G	N	I	L	L	Q	V	M	K	C	I	G	F	A	I	R	N	R	F
AAT	CAT	CGC	AGT	AAG	CAA	CAC	CCC	AAG	AGA	GAG	CAC	TGG	ATG	GAC	TGG	GCG	GAA	GAG	AAA	TAT	GAT	AAA
N	H	R	S	K	Q	H	P	K	R	E	H	W	M	D	W	A	E	E	K	Y	D	K
CTC	CTG	ATT	GCT	CAG	GTG	AAG	ATG	GTG	TTG	AAG	GTT	CTG	TTC	CTC	TAT	ATC	CCT	CTG	CCA	ATG	TTC	TGG
L	L	I	A	Q	V	K	M	V	L	K	V	L	F	L	Y	I	P	L	P	M	F	W
GCC	TTA	TTT	GAC	CAG	CAG	GGC	TCC	CGT	TGG	ACT	CTT	CAG	GCC	ACT	ACT	ATG	GAT	GGT	AAC	TTT	GGA	GCG
A	L	F	D	Q	Q	G	S	R	W	T	L	Q	A	T	T	M	D	G	N	F	G	A
TTT	ATA	ATT	CAG	CCA	GAT	CAG	ATG															
F	I	I	Q	P	D	Q	M															

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

The partial nucleotide sequences of the carp PepT1 exhibited highest sequence similarity to the zebrafish PepT1. In particular, the first cDNA gene fragment

sequence exhibited 84% similarity to zfPepT1 and over 64% with respect to the three teleosts for which sequences were made available (Table 3).

Table 3

Similarities of common carp, *Cyprinus carpio*, PepT1 gene sequences vs. other vertebrate PepT1 gene sequences (zebrafish, teleosts, birds and mammals) regarding gene nucleotides and amino acids they code for

Species	Common carp PepT1 fragment 1		Common carp PepT1 fragment 2	
	similarity of nucleotides	similarity of amino acids [%]	similarity of nucleotides	similarity of amino acids
	[%]		[%]	[%]
Zebrafish (<i>Danio rerio</i>)	84	86	87.5	92
Teleost fishes (3 species) ^a	64.5	73.5	63.5	76.5
Birds (2 species) ^b	96.5	98	97.5	97.5
Mammals (7 species) ^c	63	52	63	80.5
All species (13)	41.5	42	46	56

^azebrafish, *Danio rerio*; icefish, *Chionodraco hamatus*; Atlantic cod, *Gadus morhua*; ^bchicken, *Gallus gallus*; turkey *Meleagris gallopavo*; ^chuman, *Homo sapiens*; rabbit, *Oryctolagus cuniculus*; rat, *Rattus norvegicus*; mouse, *Mus musculus*; sheep, *Ovis aries*; pig, *Sus scrofa*; dog, *Canis lupus familiaris*.

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.

Similarly, the second cDNA fragment exhibited 87.5% similarity to the zfPepT1 gene fragment and 63.5 % similarity among the teleosts. High level of homology of this fragment was observed also in GenBank PepT1 sequences for birds and mammals, 97.5 and 63%, respectively (Tables 2, 3). The inferred amino acid sequence from this fragment spanned from TM 5 to the start of TM 8 and exhibited 92% similarity to the zebrafish PepT1 amino acid sequence (Table 5), 76.5% among other fish, bird (97.5) and mammal (80.5) species.

Table 4

Comparison of amino acid sequence of PepT1 protein for various vertebrates available from the GenBank database—fragment 1

human	89	IVYTTIGQAVTSVSSINDLTDHNDGTPDSLPHVHVLISLIGLALIALGTGGIKPCV	143
rabbit	89	IVYTTIGQAVTSLSSVNELTDNNHDGTPDSLPHVAVCMIGLLIALGTGGIKPCV	143
rat	89	IVYTTIGQAVISVSSINDLTDHHDGSPNNLPLEVALSMIGLALIALGTGGIKPCV	143
mouse	89	IVYTTIGQAVISVSSINDLTDHHDGSPDSLPHVVALSMVGLALIALGTGGIKPCV	143
sheep	89	IVYTTIGQVVIASVSSINDLTDFNHDGTPNNISVHVALSMIGLVIALGTGGIKPCV	143
pig	89	IVYTTIGQVMAVASVSSINDLTDFDHNGTPNSMSVHVALSMIGLALIALGTGGIKPCV	143
dog	89	IVYTTIGQAVTAVSSINDLTDYNKDGTPDNLVHVALSMIGLALIALGTGGIKPCV	143
chicken	95	IVYTTIGQAVMAVASVSSINDMTDQNRDGNPDNIAVHIALSMTGLILIALGTGGIKPCV	149
turkey	95	IVYTTIGQAVMAISSINDMTDQNRDGNPDNIAVHIALSVTGLILIALGTGGIKPCV	149
zebrafish	96	IVYTTIGQVINMAISAIHDTIDANRDGKPDNKLHISLSMLGLILIALGTGGIKPCV	150
icefish	15	IVYMLGQVVMMAISAIHDLTDANMDGTPDNMTLHVALSMVGLILIALGTGGIKPCV	69
atlantic cod	96	IVYTLGQVVMMAISAIHDTIDANKDGTPDNMTLHVALSMVGLILIALGTGGIKPCV	150
carp	1	---TIGQVINMAISAIHDTIDTNRDGTDPDNMTLHVALSMVGLILIALGTGGIK---	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

		←-----5-----→		←-----6-----→	
human	154	GQEKQNRFFSIFYLA	INAGSLLSTIITPMLRVQCGIHSKQACYP	PLAFGVPAAL	208
rabbit	154	GQEKQNRFFSIFYLA	INAGSLLSTIITPMVRVQCGIHVKQACYP	PLAFGIPAIL	208
rat	154	GQEKQNRFFSIFYLA	INAGSLLSTIITPILRVQCGIHSQQACYP	PLAFGVPAAL	208
mouse	154	GQEKQNRFFSIFYLA	INGGSLSTIITPILRVQCGIHSQQACYP	PLAFGVPAAL	208
sheep	154	GQEKQNRFFSIFYLA	INAGSLLSTIITPMLRVQCGIHSKQACYP	PLAFGVPAAL	208
pig	154	GQEKQNRFFSIFYLA	INAGSLLSTIITPMLRVQCGIHSTQACYP	PLAFGVPAAL	208
dog	154	GQEKQNRFFSIFYLA	INAGSLISTIVTPMLRVHECGIYSQACYP	PLAFGVPAAL	208
chicken	160	HQEKQSRFFSIFYLS	INAGSLISTIITPILRAQECGIHSRQCCYP	PLAFGVPAAL	214
turkey	160	HQEKQSRFFSIFYLS	INAGSLISTIVITPILRAQECGIHSRQCCYP	PLAFGVPAAL	214
zebrafish	161	HQEKQSTFFSIFYLS	INAGSLLSTLITPILRSQECGIYAKQSCYP	PLAFGVPAAL	215
icefish	80	HQEKQSTFFSIFYLS	INAGSLLSTVITPILRAQECGINTKQSCYP	PLAFGVPAAL	134
a. cod	161	HQEKQSTFFSIFYLS	INAGSLLSTIITPILRAQECGIYSQKQCY	PLAFGVPAAL	215
carp	1	---KQSTFFSIFYLS	INAGSLLSTLITPILRAQECGIYSKQSCYP	PLAFGVPAAL	52
-----→					
human	209	MAVALIVFVLGSGMYKKFKPQGN	IMGVAKCIGFAIKNRFRRHSKAFPKREHWD		263
rabbit	209	MAVSLIVFIIIGSGMYKKFKPQGN	ILSKVVKCICFAIKNRFRRHSKQFPKRAHWD		263
rat	209	MAVALIVFVLGSGMYKKFKPQGN	IMGVAKCIRFAIKNRFRRHSKAFPKRNHWD		263
mouse	209	MAVALIVFVLGSGMYKKFKPQGN	IMGVAKCIGFAIKNRFRRHSKAYPKREHWD		263
sheep	209	MAVSLIVFVIGSGMYKKVQPGN	IMSKVARCIGFAIKNRISHRSKKFPKREHWD		263
pig	209	MAVSLIVFVMGSRMYKKLKPQGN	VMKAVKICIGFAIKNRFRRHSKKFPKREHWD		263
dog	209	MAVSLIVFVIGSGMYKKFKPQGN	VMGVKVCIGFALKNRFRRHSKQFPKREHWD		263
chicken	215	MAVSLVVFIAAGSGMYKKVQPGN	IMVRVCKCIGFAIKNRFRRHSKEYPKREHWD		269
turkey	215	MAVSLIVFIAAGSGMYKKVQPGN	IMVQVCKCIGFAIKNRFRRHSKEYPKREHWD		269
zebrafish	216	HVVVALIVFVIGHKMYIMESPKGN	ILLQVINCIGFALNNRFRHRGKQYPKREHWD		270
icefish	135	HVVVALVVFILGSRMYIKTPQGN	IMVKVCTCIGFAVKNRFRHRSSQYPKRAHWD		189
a. cod	216	HVVVALIVFIVGSRMYTKVAPKGN	IMLEVCKCIWYAVKNRFRNRSSIPKREHWD		270
carp f2	53	HVVVALIVFIAGHMYIMESPKGN	ILLQVMKICIGFAIRNRFNRHSKQHPKREHWD		107
←-----7-----→					
human	264	WAKEKYDERLISQIKM	TRVMFLYIPLPMFWALFDQQGSRWTLQATT	MSGKIGAL	318
rabbit	264	WAKEKYDERLIAQIKM	TRVFLYIPLPMFWALFDQQGSRWTLQATT	SGRIGIL	318
rat	264	WAKEKYDERLISQIK	INTKVMFLYIPLPMFWALFDQQGSRWTLQATT	TGTGKIGTI	318
mouse	264	WAKEKYDERLISQIK	MVTKVMFLYIPLPMFWGLFDQQGSRWTLQATT	INGKIGAN	318
sheep	264	WASEKYDERLISQIK	MVTRVMFLYIPLPMFWALFDQQGSRWTLQATT	MSGKIGII	318
pig	264	WAKEKYDERLICQIK	MVTRVMFLYIPLPMFWALFDQQGSRWTLQATT	MSGQIGLL	318
dog	264	WAKEKYDERLISQIK	MVTKVMFLYIPLPMFWALFDQQGSRWTLQATT	MSGKIGLL	318
chicken	270	WASEKYDKRLIAQTK	MVLKVLFLYIPLPMFWALFDQQGSRWTLQATT	DGDFGAM	324
turkey	270	WASEKYDKRLIAQTK	MVLKVLFLYIPLPMFWALFDQQGSRWTLQATT	DGDFGAL	324
zebrafish	271	WAEKYDKLLIAQVK	MVVKVLFLYIPLPMFWALFDQQGSRWTLQATT	DGNFGGF	325

a. cod	271	WADEKYEKLLIAQIKHVLKVLFLYIPLPMFWTLFDQGSRRWTLQATTMDGDFGLL	325
icefish	190	W-----	
carp f2	108	WAEKYDKLLIAQVKHVLKVLFLYIPLPMFWALFDQGSRRWTLQATTMDGNFGAF	162
←-----8-----→			
human	319	EIQPDQMQTVNAILIVIMVPIFDVAVLYPLIAKCGNFNTSLKKMAVGMFLASMAFV	373
rabbit	319	EIQPDQMQTVNTILIIILVPIMDAVVYPLIAKCGLNFTSLKKMTIGMFLASMAFV	373
rat	319	EIQPDQMQTVNAILIVIMVPIVDVAVVYPLIAKCGNFNTSLKKMTVGMFLASMAFV	373
mouse	319	EIQPDQMQTVNAILNVNNGPNVDVAVVRSIAKCGNFNTSLKKMTVGMFLASMAFV	373
sheep	319	EIQPDQMQTVNAILIVVMVPIVDVAVVYPLIAKCGLNFTSLKKMTVGMFLASMAFV	373
pig	319	KIQPDQMQTVNAILIVIMVPIMDAVVYPLIAKCGLNFTSLRKMTVGMFLASMAFV	373
dog	319	EVQPDQMQTVNAILIVVMVPIMDAVVYPLIAKCGNFNTSLRKMTVGMFLASMAFV	373
chicken	325	QIQPDQMQTVNPILIIIMVPPVDAVIYPLIQCKINFNTPLRKITVGMFLAGLAFV	379
turkey	325	QIQPDQMQTVNPILIIIMVPPVDAVIYPLIQCKINFNTPLRKITVGMFLAGLAFV	379
zebrafish	326	VIQPDQMQIVNPILIVIMVPIIMDSAIYPLIKLCRINFNTPLRKMTVGMFLAALAFV	380
a. cod	326	VIQPDQMQTVNPILILGFVPIVDSVIYPLIAKCGLNFTPLRKMTVGMFLAALAFV	380
carp f2	163	IIQPDQM-----	169

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

Table 6

Similarity of amino acid sequences in various species with division into transmembrane domains and connecting loops

Species	Similarity of amino acid [%]				
	TM 5 18 aa	Loop 5/6 28 aa	TM6 19 aa	Loop 6/7 45 aa	TM 7 18 aa
Mammals ^a	100	64	74	71	89
Birds ^b	100	96.5	95	98	100
Fishes ^c	100	78.5	84	46.5	78
Zebrafish/carp ^c	100	89	88.5	80	94.5
All species	72	46.5	63	44.5	61

^ahuman, *Homo sapiens*; rabbit, *Oryctolagus cuniculus*; rat, *Rattus norvegicus*; mouse, *Mus musculus*; sheep, *Ovis aries*; pig, *Sus scrofa*; dog, *Canis lupus familiaris*; ^bchicken, *Gallus gallus*; turkey *Meleagris gallopavo*; ^czebrafish, *Danio rerio*; icefish, *Chionodraco hamatus*; Atlantic cod, *Gadus morhua*.

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).

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.

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