Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2012; 37 (2) ; 204–211.
doi: 10.5505/tjb.2012.95914

Research Article [Araştırma Makalesi]

Yayın tarihi 15 Nisan, 2012 © TurkJBiochem.com

[Published online 15 Nisan, 2012]

Peptide transporter Pept1 in *Cyprinus carpio* L.'s intestine: cDNA cloning and sequence analysis

[*Cyprinus carpio* L. bağırsağındaki peptit transporter Pept1: cDNA klonlama ve sekans analizi*]

Guoxing Nie[1],
Xiao Yan[1],
Junli Wang[1],
Hong Ming[2],
Bei Wang[1],
Junlin Zheng[1],
Xuejun Li[1],
Xianghui Kong[1]

[1]College of Life Sciences, Henan Normal University, Xinxiang, Henan, China
[2]Department of Life Sciences and Technology, Xinxiang Medical University, Xinxiang, Henan, China

Yazışma Adresi
[Correspondence Address]

Prof. Dr. Guoxing Nie

College of Life Sciences, Henan Normal University
46# Jianshe Road, Muye District, Xin Xiang, 453007 China.
Phone: +86-0373-3329129
Fax: +86 -0373-3329102
E-mail: niegx@htu.cn

*** Translated by [çeviri]** Ebru Karabal

Registered: 30 September 2011; Accepted: 23 February 2012
[Kayıt Tarihi: 30 Eylül 2011; Kabul Tarihi: 23 Şubat 2012]

ABSTRACT

Aim: To clone the *Pept1* gene coding peptide transporter from the intestine of *Cyprinus carpio* L. and analyze its sequence.

Material and Methods: A full-length cDNA encoding the Pept1 of *Cyprinus carpio* L. was cloned using RT-PCR and Rapid Amplification of cDNA Ends methods. We compared amino acid sequence of Pept1 with that of other 17 species and the sequences homology was analyzed using the Laser-gene analysis software package (DNAMAN 6.0, USA). The 3-D structure of the protein was predicted using comparative protein modeling program SWISS-MODEL.

Results: The results showed that *Cyprinus carpio* L. *Pept1* with 3118 bp was composed of 127 bp 5'-untranslated region, 819 bp 3'-untranslated region, and 2172 bp open reading frame. *Pept1* could encode 723 amino acids with molecular weight of approximately 81.1 kDa and isoelectric point of 5.67. The predicted amino acid sequence has the highest similarity with that of zebra fish (80.9%), and the lowest similarity with that of sea urchin (40.5%). Twelve transmembrane domains were predicted in the 3-D protein model. Tyr-175 located in the fifth transmembrane domain and His-65 situated in the second transmembrane domain were essential for the catalytic activity of Pept1.

Conclusion: The *Cyprinus carpio* L. *Pept1* with 2172 bp open reading frame encodes 723 amino acids. The Pept1 contains 31.81% of *α*-helix, 23.65% of extended strand, 3.60% of *β*-turn, 40.94% of random coil and twelve transmembrane domains. *Pept1* gene has a highly conserved sequence.

Key Words: *Pept1* gene; cDNA; sequence analysis; protein tertiary structure; *Cyprinus carpio* L.

Conflict of interest: There is no conflict of interest in respect of this manuscript.

ÖZET

Amaç: *Cyprinus carpio* L. bağırsağından peptit transporter kodlayan *Pept1* genini klonlamak ve sekansını analiz etmek.

Gereç ve Yöntemler: *Cyprinus carpio* L.'den *Pept1*'i kodlayan tüm uzunluktaki cDNA, RT-PCR ve cDNA Sonlarının Hızlı Amplifikasyonu (*Rapid Amplification of cDNA Ends* – RACE) metodları ile klonlandı. Pept1 amino asit sekansı 17 tür ile karşılaştırıldı ve Laser-gen analizi yazılımı (DNAMAN 6.0, ABD) aracılığıyla sekans homolojisi analiz edildi. Karşılaştırmalı protein modelleme programı SWISS-MODEL kullanılarak proteinin tahmini üç boyutlu yapısı oluşturuldu.

Bulgular: Çalışma bulgularına göre, 3118 baz çifti bulunan *Cyprinus carpio* L. *Pept1* geninin 5'-transle edilmeyen bölgesinde 127 baz çifti, 3'-transle edilmeyen bölgesinde ise 819 baz çifti bulunmakta ve açık okuma alanı 2172 baz çiftinden oluşmaktadır. *Pept1*, moleküler ağırlığı yaklaşık 81.1 kDa ve izoelektrik noktası 5.67 olan 723 amino asit kodlayabilmektedir. Tahmini amino asit sekansı, zebra balığı ile en yüksek (%80.9), denizkestanesi ile de en düşük (%40.5) benzerliği göstermektedir. Üç boyutlu protein modeline göre 12 transmembran bölge olduğu tahmin edilmektedir. Pept1'in katalitik aktivitesi için beşinci transmembran bölgede bulunan Tyr-175 ve ikinci transmembran bölgede bulunan His-65'in gerekli oldukları belirlenmiştir.

Sonuçlar: Açık okuma alanı 2172 baz çiftinden oluşan *Cyprinus carpio* L. *Pept1* geni, 723 amino asit kodlamaktadır. Pept1, %31.81 *a*-sarmalı, %23.65 açık şerit, %3.60 *b*-dönüşü, %40.94 rastgele sarım ve 12 transmembran bölgeden oluşmaktadır. *Pept1* geninin yüksek oranda korunmuş sekansı bulunmaktadır.

Çıkar Çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Anahtar Kelimeler: *Pept1* geni, cDNA, sekans analizi, protein üçüncül yapısı, *Cyprinus carpio* L.

Introduction

Cell membrane compartmentalizes metabolic processes which functions as a selective barrier for permeation of nutrients and xenobiotics, so transport of nutrients across the plasma membrane is a critical step in nutrient homeostasis. Maintenance of an intracellular environment which is different from the extracellular environment is essential to life, and therefore a large spectrum of membrane proteins with highly specialized functions has emerged during evolution [1].

Peptide transport is a specific biochemical process in which small peptides are transported across membrane by energy-dependent carriers. A large number of genes which encode components of oligopeptide transport systems in bacteria have been cloned and sequenced, although only a few eukaryotic peptide transport gene sequences have been reported. Cloning and sequence analyses of the cDNAs encoding the mammalian oligopeptide transporters [2-7] have offered insight into the molecular mechanism for the uptake of oligopeptides. They have been identified as proton-dependent transporters with physiological roles to absorb small peptides arising from digestion of dietary protein in the small intestine [8] and peptides generated by luminal peptidases in the kidney [9,10].

Peptide transporters belong to the SLC15 family [11]. Recently, at least five distinct peptide transporters have been found [12]: peptide transporter 1 (Pept1), peptide transporter 2 (Pept2), peptide transporter 3 (PTR3), peptide/histidine transporter 1 (PHT1) and human peptide transporter 1 (HPT1). Pept1 and Pept2 are most widely studied. They are proton-driven transporters distributed in both eukaryotes and prokaryotes, using the inwardly directed proton (H^+) electrochemical gradient to drive the uptake of peptides across cell membranes [1,13]. Daniel and Kottra showed that Human Pept1 is found predominantly in the small intestine, whereas Pept2 is found in the kidney, lungs and central nervous system [14]. Twelve transmembrane (TM) helices are predicted in Pept1 and Pept2 and both N- and C-termini facing the cytoplasm, as is typical for major facilitator superfamily (MFS) members [3,15]. Pept1 operates as a high-capacity, low-affinity transporter and is the main route for dietary peptide uptake, whereas Pept2 is a low-capacity, high-affinity transporter, which is thought to mediate more selective transport in kidney and other tissues [14,16-18]. In addition to peptides, the human Pept transports a lot of orally administered drugs, including the β-lactam antibiotics [19-21] and antivirals improving the pharmacokinetic properties such as valacyclovir [22] and the vasopressor midodrine [23].

Here we report the cloning and sequence analysis of a peptide transporter Pept1 expressed in *Cyprinus carpio* L.'s intestine. The aim of this study is to investigate the molecular mechanism of peptide transport so that it can be used for further studies on protein expression and specific antibody preparation.

Material and methods

Fish acclimation

Cyprinus carpio L. (12.6 ±0.38 g) were acclimated in a 200-L tank filled with dechlorinated water at 26.8 ±0.68°C with constant aeration (DO: 6.2 ±0.2 $mg \cdot L^{-1}$) and a 12/12 h light/dark photoperiod. The fish were fed for four times each day (8:30 am, 11:30 am, 14:30 pm and 17:30 pm) with commercial pellet feed. After the acclimation, ten fish were randomly chosen for experiments. The fish were dissected after general anesthesia, intestines were obtained and the contents in guts were cleared rapidly. All the operations were conducted under aseptic conditions on an ice-bath. The Ethics Committee of the Second Affiliated Hospital of Xinxiang Medical University (No.2011026; March 5, 2011) approved the use of fish and the experimental protocols in this study.

Total RNA extraction and RT-PCR

The intestines were quickly frozen in liquid nitrogen and used to extract the total RNA with TRIzol reagent (purchased from Invitrogen). cDNA was synthesized starting from 5 mg total RNA using AMV reverse transcriptase (from Shanghai Sangon) and oligo-p $(dT)_{18}$ Primer (from Shanghai Sangon) in a 20 µL reaction, according to the manufacturer's instructions. The Pept1 cDNAs were then amplified by PCR in a total volume of 50 µL, containing 5 µL template cDNA, 10 mM Tris-HCl (pH 9.0), 0.2 mM dNTPs, 1.25 mM $MgCl_2$, 50 mM KCl, 40 pmol of each primer, and 1 unit of Taq polymerase (from Takara, Japan). PCR primers are listed in Table 1. An initial denaturation of 3 min at 94°C was followed by 35 cycles (1 cycle: 50s at 94°C, 50s at 60°C and 2 min at 72°C), and a final extension for 10 min at 72°C. The annealing temperatures of PCR reaction varied according to the *Pept1* to be amplified (Table 1). The PCR products were size-fractionated by 1 % agarose gel electrophoresis. Photographs of the gels stained with ethidium bromide revealed an inverted black/white format (Fig. 1).

Cloning of Pept1 cDNA from Cyprinus carpio L.'s intestine

The amplified bands corresponding to Pept1 cDNA were accurately excised from the 1 % agarose gel and purified using the gel extraction kit (from Takara, Japan). The purified *Pept1* cDNAs were ligated into the pGEM-T Easy vector (Promega, USA), and the resultant recombinant plasmids were transformed into competent *Escherichia coli* strain JM109. For each cDNA, 4-6 plasmid clones containing Pept1 cDNAs were sequenced by ABI3730 using M13+/- universal primers (Takara, Japan).

Results

Isolation of the *Cyprinus carpio* L. *Pept1* cDNA by Rapid Amplification of cDNA Ends (RACE) method

The primers were originally designed from highly conserved regions of *Pept1* based on the sequence

Table 1. Sequences of oligonucleotide primers used for PCR and Rapid Amplification of cDNA Ends (RACE).

Names	Oligonucleotide sequence (5'→3')	Length (bp)
3' GSP1 Pept1	GACTCGTGGCTGGGAAAGTTCAA	22
3' GSP2 Pept1	CTGTGTGGCAGCGTTTGGAGGAG	23
3' RACE outer	TACCGTCGTTCCACTAGTGATTT	23
3' RACE inner	CGCGGATCCTCCACTAGTGATTTCACTATAGG	32
5' GSP1 Pept1	AGTATAGGGTTCACGATCTGCATC	24
5' GSP2 Pept1	TGGGTGTGATGAGAGTGGAGAGAAG	25
5' RACE outer	CATGGCTACATGCTGACAGCCTA	23
5' RACE inner	CGCGGATCCACAGCCTACTGATGATCAGTCGATG	34

Note: The primers were based on the *Pept1* sequences of other animals deposited in GenBank.

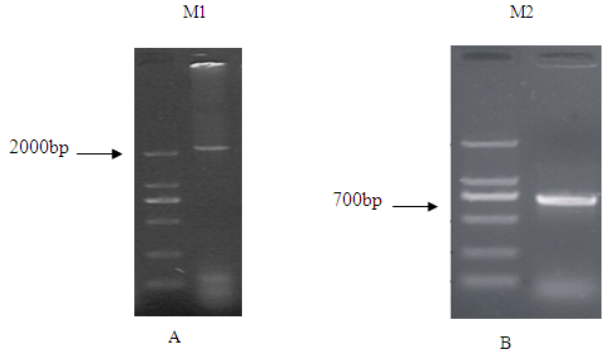

Figure 1. The results of RACE-PCR to amplify *Pept1*. A, the result of 5'-RACE, the amplified gene fragment was ~2200 bp; B, the result of 3'-RACE, the amplified gene fragment was ~700bp; M1, 2000 bp DNA ladder; M2, 1000bp DNA ladder.

alignment of zebra fish (*Danio rerio*), turkey (*Meleagris gallopavo*), salmon (*Salmo salar*), rock fish (*Sebastes nebulosus*), brown rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*), pig (*Sus scrofa*), *Perca* (*Perca flavescens*), mouse (*Mus musculus*), *Macaca* (*Macaca fascicularis*), human (*Homo sapiens*), Atlantic cod (*Gadus morhua*), cattle (*Bos taurus*), bass (*Dicentrarchus labrax*), dog (*Canis lupus familiaris*), sheep (*Ovis aries*), and sea urchin (*Strongylocentrotus purpuratusand*) *Pept1* cDNA sequences from GenBank. Employing the RACE strategy, the full-length *Pept1* of *Cyprinus carpio* L. was cloned. The 5'-RACE and 3'-RACE results were sequenced and spliced to obtain the full-length cDNA (Fig 1). The complete coding sequence of the *Cyprinus carpio* L. *Pept1* cDNA with 3118 nucleotides comprised a coding sequence region with a 2172 bp open reading frame (ORF), a 127 bp 5'-untranslated region and a 819 bp 3'-untranslated region, including poly (A).

Sequence analysis of Cyprinus carpio L. *Pept1 gene*

The deduced amino acid sequence of *Cyprinus carpio* L. Pept1 using EXPASY is composed of 723 amino acids with a molecular weight of approximately 81.1 kDa and the isoelectric point of 5.67 (Fig. 2). The secondary structure of the deduced Pept1 amino acid sequence was analyzed to seek potential TM regions using TMHMM Server v.2.0 (DTU) (Fig. 3). Twelve putative TM domains were identified using SOPMA. The Pept1 contains 31.81 % of α-helix, 23.65 % of extended strand, 3.60 % of β-turn and 40.94 % of random coil (Fig. 4).

Homology and phylogenetic analysis of Pept1 gene

The *Cyprinus carpio* L. *Pept1* cDNA sequence obtained in this study has been submitted to GenBank and assigned the accession number AEX13747.1. Alignment and comparison of the deduced amino acid sequence of the *Cyprinus carpio* L. Pept1 to those already characterized in vertebrates were performed in order to recognize and recapitulate major structural/functional features common to vertebrate Pept1. The deduced amino acid sequence of *Cyprinus carpio* L. Pept1 was 80.9 %, 62.4 %, 68.7 %, 63.4 %, 60.0 %, 60.2 %, 61.0 %, 65.1 %, 58.9 %, 59.9%, 59.9 %, 66.6 %, 60.9 %, 68.7 %, 60.5 %, 60.9 %, and 40.5%identical to zebra fish (*Danio rerio*), turkey (*Meleagris gallopavo*), salmon (*Salmo salar*), rock fish (*Sebastes nebulosus*), brown rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*), pig (*Sus scrofa*), *Perca* (*Perca flavescens*), mouse (*Mus musculus*), *Macaca* (*Macaca fascicularis*), human (*Homo sapiens*), Atlantic cod (*Gadus morhua*), cattle (*Bos taurus*), bass (*Dicentrarchus labrax*), dog (*Canis lupus familiaris*), sheep (*Ovis aries*), and sea urchin (*Strongylocentrotus purpuratusand*), respectively. The homology among the sequences was calculated using the Laser-gene analysis software package (DNAMAN 6.0, USA) (Fig. 5). The deduced amino acid sequence of Pept1 in *Cyprinus carpio* L. was least similar with that of sea urchin (40.5 %) and highest similar with that of zebra fish (80.9 %). Overall, the phylogenetic reconstruction of the vertebrate Pept1 tree suggested substantially higher sequence divergence among the proteins of the fish group than among those of the mammalian group.

```
-127                                                            GAAAACA
-120 GCTTTGTCTGTCCCGGGTAGTGTGTGAGTGAGTGTGTGAGTGAGTGTGAGTGTGCATCTC
 -60 TAAACTGAAGGATATTCTTAACGAGAGGACTAGAAGGCAGCCTCAGTCAAAGCAGTCATC
   1 ATGGCAGATGAGGAGAGAAATACAAAGAAGGCCAAGAATAGGCCAAGTTGCTTGGGATAT
   1  M  A  D  E  E  R  N  T  K  K  A  K  N  R  P  S  C  L  G  Y
  61 CCTGTCAGTATATTTTTCATTGTGGTGAATGAGTTCTGTGAGCGATTCTCCTACTATGGC
  21  P  V  S  I  F  F  I  V  V  N  E  F  C  E  R  F  S  Y  Y  G
 121 ATGAAAGCGGTGCTGGTGCTGTACTTCAAGTACTTTATCGGTTGGGACAATGATTTGTCC
  41  M  K  A  V  L  V  L  Y  F  K  Y  F  I  G  W  D  N  D  L  S
 181 ACAACCATCTACCACACGTTCGTGGCCCTGTGCTACCTAACACCTATCCTGGGGGCCATC
  61  T  T  I  Y  H  T  F  V  A  L  C  Y  L  T  P  I  L  G  A  I
 241 ATCGCAGACTCATGGCTGGGCAAGTTCAAGACTATCGTGTATCTGTCTATAGTGTACACC
  81  I  A  D  S  W  L  G  K  F  K  T  I  V  Y  L  S  I  V  Y  T
 301 ATCGGCCAGGTGATCATGGCAATCAGTGCCATACATGACATCACAGATACAAACAGAGAC
 101  I  G  Q  V  I  M  A  I  S  A  I  H  D  I  T  D  T  N  R  D
 361 GGTACACCTGACAACATGACATTCCATACTGCCATGTCCATGCTGGGACTCATCCTCATT
 121  G  T  P  D  N  M  T  F  H  T  A  M  S  M  L  G  L  I  L  I
 421 GCTTTGGGAACTGGAGGTATTAAACCCTGTGTGGCAGCGTTTGGAGGAGATCAGTTTGAA
 141  A  L  G  T  G  G  I  K  P  C  V  A  A  F  G  G  D  Q  F  E
 481 GAACATCAGGAAAAACAAAGAAGTACTTTCTTTTCCATATTTTATCTGTCCATTAATGCT
 161  E  H  Q  E  K  Q  R  S  T  F  F  S  I  F  Y  L  S  I  N  A
 541 GGCAGTCTTCTCTCCACTCTCATCACACCCATCCTGAGAGCTCAAGAGTGTGGTATCTAT
 181  G  S  L  L  S  T  L  I  T  P  I  L  R  A  Q  E  C  G  I  Y
 601 TCAAAGCAAAGTTGTTTCCCGCTGGCTTTTGGGGTCCCTGCCGCTCTCATGGTGGTTGCC
 201  S  K  Q  S  C  F  P  L  A  F  G  V  P  A  A  L  M  V  V  A
 661 CTCATTGTGTTCATCGCAGGCCACAACATGTACATTATGGAATCCCCCAAAGGCAACATC
 221  L  I  V  F  I  A  G  H  N  M  Y  I  M  E  S  P  K  G  N  I
 721 CTGCTGCAAGTCATGAAGTGCATTGGATTTGCAATTAGGAACCGTTTCAATCATCGCAGT
 241  L  L  Q  V  M  K  C  I  G  F  A  I  R  N  R  F  N  H  R  S
 781 AAGCAACACCCCAAGAGAGAGCACTGGATGGACTGGGCGGAAGAGGAATATGATAAACTC
 261  K  Q  H  P  K  R  E  H  W  M  D  W  A  E  E  E  Y  D  K  L
 841 CTGATTGCTCAGGTGAAGATGGTGTTGAAGGTTCTGTTCCTCTATATCCCTCTGCCAATG
 281  L  I  A  Q  V  K  M  V  L  K  V  L  F  L  Y  I  P  L  P  M
 901 TTCTGGGCCTTATTTGACCAGCAGGGCTCCCGTTGGACTCTTCAGGCCACTACTATGGAT
 301  F  W  A  L  F  D  Q  Q  G  S  R  W  T  L  Q  A  T  T  M  D
 961 GGTAACTTTGGAGCGTTTATAATTCAGCCAGATCAGATGCAGATTGTGAACCCCATACTG
 321  G  N  F  G  A  F  I  I  Q  P  D  Q  M  Q  I  V  N  P  I  L
1021 ATTGTGATAATGGTGCCCATAATGGACTCTGCAGTGTATCCACTTATCAAGAAGTGTGGT
 341  I  V  I  M  V  P  I  M  D  S  A  V  Y  P  L  I  K  K  C  G
1081 CTTAACTTCACGCCCCTGAGGAGGATGACTGTTGGTATGCCGCTGGCCTCGTTAGCATTT
 361  L  N  F  T  P  L  R  R  M  T  V  G  M  P  L  A  S  L  A  F
1141 GTGGCTGCTGCTCTTTTGCAGATTCAAATTGACCGAACAGTCCCCAACTTCCCATCAAGC
 381  V  A  A  A  L  L  Q  I  Q  I  D  R  T  V  P  N  F  P  S  S
1201 TCTCAAACCCAGGTGAAGTTCCTGAACTTGGAGAACACACCAGTGCCTGTTGTAGTGGAG
 401  S  Q  T  Q  V  K  F  L  N  L  E  N  T  P  V  P  V  V  V  E
1261 GGGCAAGAACCGTTTGTTGTTCCTGGTTTTAATTCCTCTAATAATTATATGACACTGGAC
 421  G  Q  E  P  F  V  V  P  G  F  N  S  S  N  N  Y  M  T  L  D
1321 ACTGAGAACGTTACGGTCTCTGCTGGAGGAAGAGACGCTACAGCTTACTTCCAGAAGGAA
 441  T  E  N  V  T  V  S  A  G  G  R  D  A  T  A  Y  F  Q  K  E
1381 TCAAGGCACACGGTTTTGATAAATGCTGACAGAATGCTACAGACTCTCAATGACATAACT
 461  S  R  H  T  V  L  I  N  A  D  R  M  L  Q  T  L  N  D  I  T
1441 GAGAAACCCAATCAAGGCATGAATGCGATCAGGTTTGTGAATGGCTTTAGGTCTCATCTG
 481  E  K  P  N  Q  G  M  N  A  I  R  F  V  N  G  F  R  S  H  L
1501 AACTTCTCAGTAAAGTCTGATCATCTGGGTCCCATCGCTCCCTTACAAGCATCAGACTAC
 501  N  F  S  V  K  S  D  H  L  G  P  I  A  P  L  Q  A  S  D  Y
1561 TTCAGTGTTACCCACGGAAAAGCCAGTTTTACTATTGAAAACGAGGAAGGACAGACGTGT
 521  F  S  V  T  H  G  K  A  S  F  T  I  E  N  E  E  G  Q  T  C
1621 CACTACATCCTGCAGCTGGGCTTCGGAAGCTCCTACACAGTCCTGATCCCAAACACCTTT
 541  H  Y  I  L  Q  L  G  F  G  S  S  Y  T  V  L  I  P  N  T  F
1681 ACTTTTGGAGAATCTTGTACCGAGAACATTAAAGCAATCCAGGATATTAACCCGAACGAC
 661  T  F  G  E  S  C  T  E  N  I  K  A  I  Q  D  I  N  P  N  D
1741 ATCCACATGGCCTGGCAGGTTATTCAGTACTTTTTGATGACCTGCGGAGAAGTGGTCTTC
 581  I  H  M  A  W  Q  V  I  Q  Y  F  L  M  T  C  G  E  V  V  F
1801 TCTGTCACCGGCCTGGACTTCTCCTATTCACAGGCACCAAGCAATATGAAGTCAGTGCTC
 601  S  V  T  G  L  D  F  S  Y  S  Q  A  P  S  N  M  K  S  V  L
1861 CAGGCTGGTTGGCTGTTGACTGTTGCAGTGGGGAACATCATCGTGCTCATTGTGGCTGAG
 621  Q  A  G  W  L  L  T  V  A  V  G  N  I  I  V  L  I  V  A  E
1921 GCAGGCTCACTTCCAGAGCAGTGGGCTGAATACGTGCTGTTTGCATGTCTCCTGGTGGCT
 641  A  G  S  L  P  E  Q  W  A  E  Y  V  L  F  A  C  L  L  V  A
1981 GTGAGTATCATCTTTGCGGTCATGGCTTACTTCTACACCTACATGGATCCGGCAGAAATT
 661  V  S  I  I  F  A  V  M  A  Y  F  Y  T  Y  M  D  P  A  E  I
2041 GAGGCTAAATTTATGGAGCTCGAGCCAGATGATAAGAAAAGGAAAGAGCTTGAAATGATG
 681  E  A  K  F  M  E  L  E  P  D  D  K  K  R  K  E  L  E  M  M
2101 ACGAAAGACAATTTGGCATATGTTAACAACGAGACGTCAAACAACACAGATGTCAAACAA
 701  T  K  D  N  L  A  Y  V  N  N  E  T  S  N  N  T  D  V  K  Q
2161 ACGAAGATCTAGATGCATCGCTTCTTTTTTCTCTTGATCGGTGGTTTGAAAGTGCTGGTG
 721  T  K  I  *
2221 TTCTCTTTCCTATTGACTGAAGAACTGATTCACCATTTTTTTTTTTATCAAAGCTGGGCT
2281 TGGGATTTGTTATGCAGTAGCAAAAATCAGCCCAAAAAAAGCTAATTCTACCTGAAACTG
2341 AATGGTGTGTTTTGGGACGACAACCATGGGCAGAAGGTTTGGTAGTTTAATAGAGCATGA
2401 TATGGTTAAGATCAGTGTTAAAGTGACCGTGCGTTGGTGCCTTAGCAGGCGGGTTTTATT
2461 TATGTGTTACTATCAATAAATAAATAAGTGAAAAAAAAAGAGCAAGTGACGCTCTTTTAT
2521 GGAAAATGCAATTTACCATTATAATGAAAAACCAAATTAAATGGAAACCATAGAGTGGAA
2581 GTACATCATCACACTGCTATTTCAAGCAACACAGTCCTGTTTGTGTAATTGTGGATTGGA
2641 TCCTTCAGAAAATATTCTAAAGTGCCTCTAAATAATTTTGATTAATAGCCATTCATCTAC
2701 ACCAAGTAATGGGTAAAACAAGGTATTGTAAAGTTTTCTTTGAAGTAATTCATTTTAATG
2761 CTGTTATTCTGCAACTTAAAATCTGCATTATTTGAAGGGGTTACAATAAGAATGTACCAG
2821 ATAATGAGCATTAGAGTGCAGTACTTTCCTCTATGTATTTTTAAATTATTTTGTTTCTAC
2881 ATAACACTGTATATGGATTTTAAACTACACTGGGTCCAATAAGCTATGATTAAATCAACT
2941 TTTGATGAAGTGCTTCAATAAAAAAATATATTTATGCCTCAAAAAAAAAAA
```

Figure 2. Nucleotide and deduced amino acid sequence of Pept1 from intestinal cells of *Cyprinus carpio* L. The sequence contains a single open reading frame which encodes a protein with 723 amino acids. The complete 5'-untranslated region was 127 nucleotides.

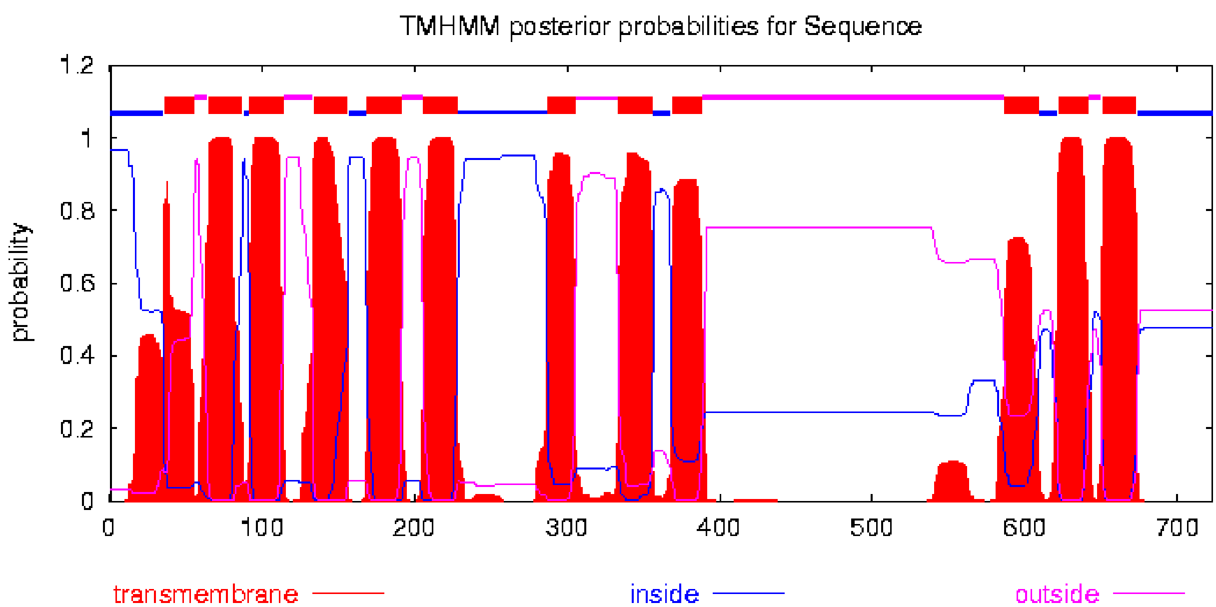

Fig. 3. Secondary structure model and predicted TM domains of Pept1 predicted by TMHMM Server v. 2.0. Both N-terminal and C-terminal contact the cytoplasm.

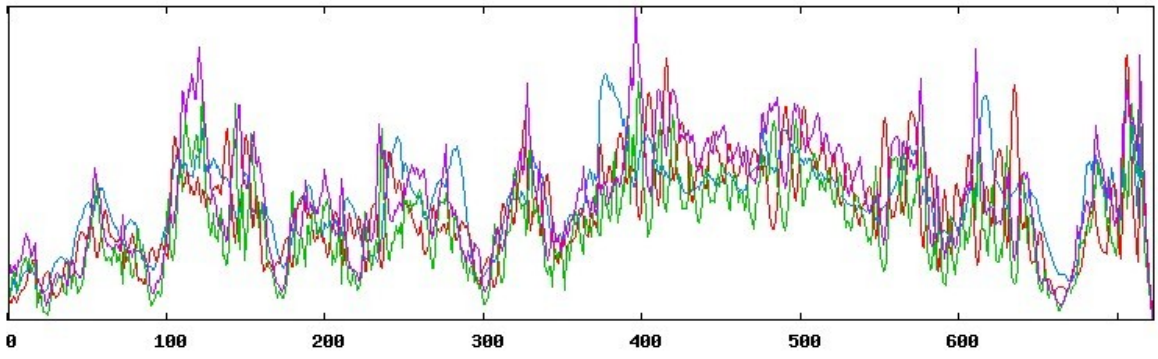

Figure 4. SOPMA result for Pept1 from *Cyprinus carpio* L.'s intestine. The Pept1 contains 31.81%of α-helix (blue), 23.65%of extended strand (red), 3.60%of β-turn (green) and 40.94%of random coil (yellow).

Phylogenetic trees were constructed using MEGA 5.0 for further examination of the evolutionary relationships among Pept1 homologs (Fig. 6).

To gain insight into the structure of the *Cyprinus carpio* L. Pept1, the structure of Pept1 was modeled. The 3-D protein models in this study were predicted by the comparative protein modeling program SWISS-MODEL (Fig. 7).

Discussion

The plasma membrane transport proteins belonging to the SLC15 family [14] is a phylogenetically conserved family which functions as integral membrane proteins for the uptake of di- and tripeptides. Up to date, many SLC15 proteins in yeast, animals and higher plants have been well characterized [24]. Pept1 serves as a low affinity but high-capacity system and is highly expressed in the small intestine with lower expression levels in the kidney and bile duct epithelium [25]. The physiological role of Pept1 is restricted to the absorption of bulk quantities of amino acids in their peptide-bound form in the small intestine.

Recently both *in vitro* and *in vivo* experimental studies have shown that di-peptide uptake is dependent on proton (H^+) electrochemical gradient flow, and therefore functions as a H^+/Peptide co- transporter [3]. The present study lays a foundation for delineating the molecular evolution and nutritional characteristics between *Cyprinus carpio* L. and other fish strains or mammals.

The Pept1 subunit of *Cyprinus carpio* L. was similar with that of zebra fish. The amino acid sequences of Pept1 from several species were classified into two major groups. The Pept1 subunits of mammals and turkey were clustered into one group. The Pept1 subunit of *Cyprinus carpio* L., zebra fish, rock fish, salmon, gadus, bass and perca were clustered into another group and sea urchin was clustered as alone. The phylogenetic reconstruction of the vertebrate Pept1 tree suggested substantially higher sequence divergence among the proteins of the fish group than among those of the mammalian group. As a conclusion, based on the characteristic analysis of the TM and cytoplasmic domains, Pept1 of *Cyprinus carpio* L. is highly conserved.

Pept1 is a complete membrane protein. There are twelve predicted TM regions, and there is a large extracellular loop between TM 9 and 10. Both N and C terminals contact the cytoplasm [26,27] (Fig. 3).

In the tertiary structure, a central cavity and a small extracellular cavity can be observed, and both of them are hydrophilic. The central cavity is located within the centre of the membrane and closed to the extracellular space by a gate made of helices TM1, TM2, TM7 and TM8, which pack closely together [11]. The binding site is formed by residues from helices TM1, TM2, TM4 and TM5 from the N-terminal six-helix bundle and from helices TM7, TM8, TM10 and TM11 from the C-terminal bundle [11]. The predicted TM regions are highly conserved, but amino acid homology within the extracellular loop is lower [27]. Fei *et al.* [3] reported that histidine residues are required for the catalytic activity of human Pept1 (hPept1). His-57 and His-121 are located within the second and fourth TM domains. Yeung *et al.* [28] has shown that Tyr-167 (Y167) in TM5 has an effect on the activity of the Pept1 transportation. The mutant in Tyr-167 does not absorb glycyl sarcosine (Gly Sar), indicating that the site Tyr-167 is required for the Pept1 activity. Similarly, in this study, Tyr-175 is found in the fifth putative TM domain. His-57 in Pept1 was found to be essential for catalytic activity because the corresponding mutants had no detectable peptide transport activity. His-57 in hPept1 is located in an almost identical topological position in both transporters, near the extracellular surface of the second putative TM domain [29]. Similarly, in this study, His-65 is located near the extracellular surface of the second putative TM domain. Sequence alignment indicated that these regions were well conserved in Pept1 (Fig. 5).

Ethical Considerations

The Ethics Committee of the Second Affiliated Hospital of Xinxiang Medical University (No.2011026; March 5, 2011) approved the use of fish and the experimental protocols in this study.

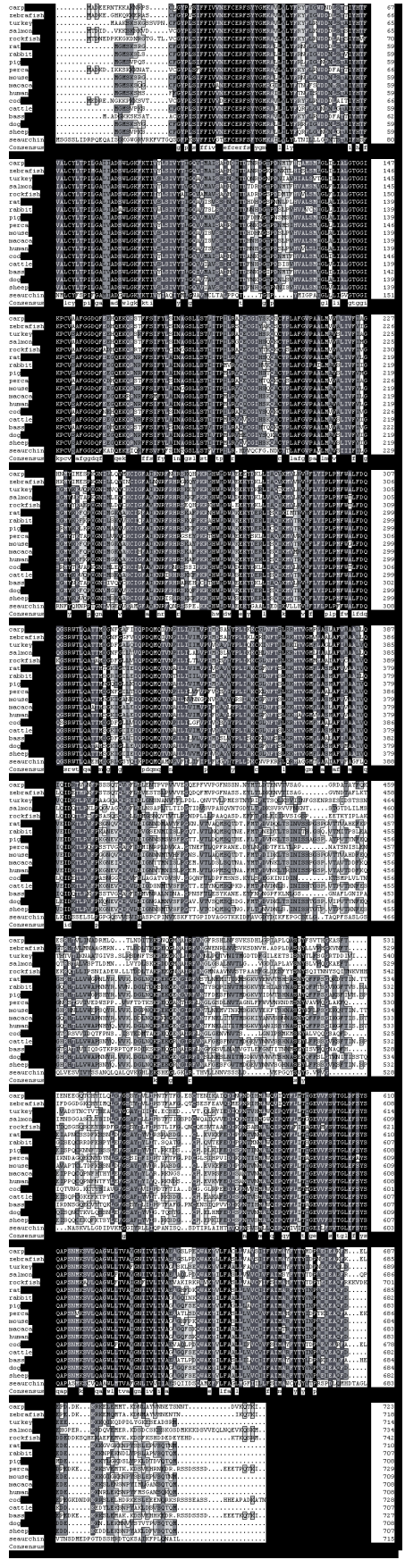

Figure 5. Alignment of deduced amino acid sequences of the Peptl subunit from the zebra fish (*Danio rerio*), turkey (*Meleagris gallopavo*), salmon (*Salmo salar*), rock fish (*Sebastes nebulosus*), brown rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*), pig (*Sus scrofa*), *Perca* (*Perca flavescens*), mouse (*Mus musculus*), *Macaca* (*Macaca fascicularis*), human (*Homo sapiens*), Atlantic cod (*Gadus morhua*), cattle (*Bos taurus*), bass (*Dicentrarchus labrax*), dog (*Canis lupus familiaris*), sheep (*Ovis aries*) and sea urchin (*Strongylocentrotus purpuratusand*). These protein sequences were aligned using the DNAMAN 6.0. Identical amino acids are shown on a black background, ≥75%similar amino acids on a gray background and ≥50%similar amino acids on a light gray background.

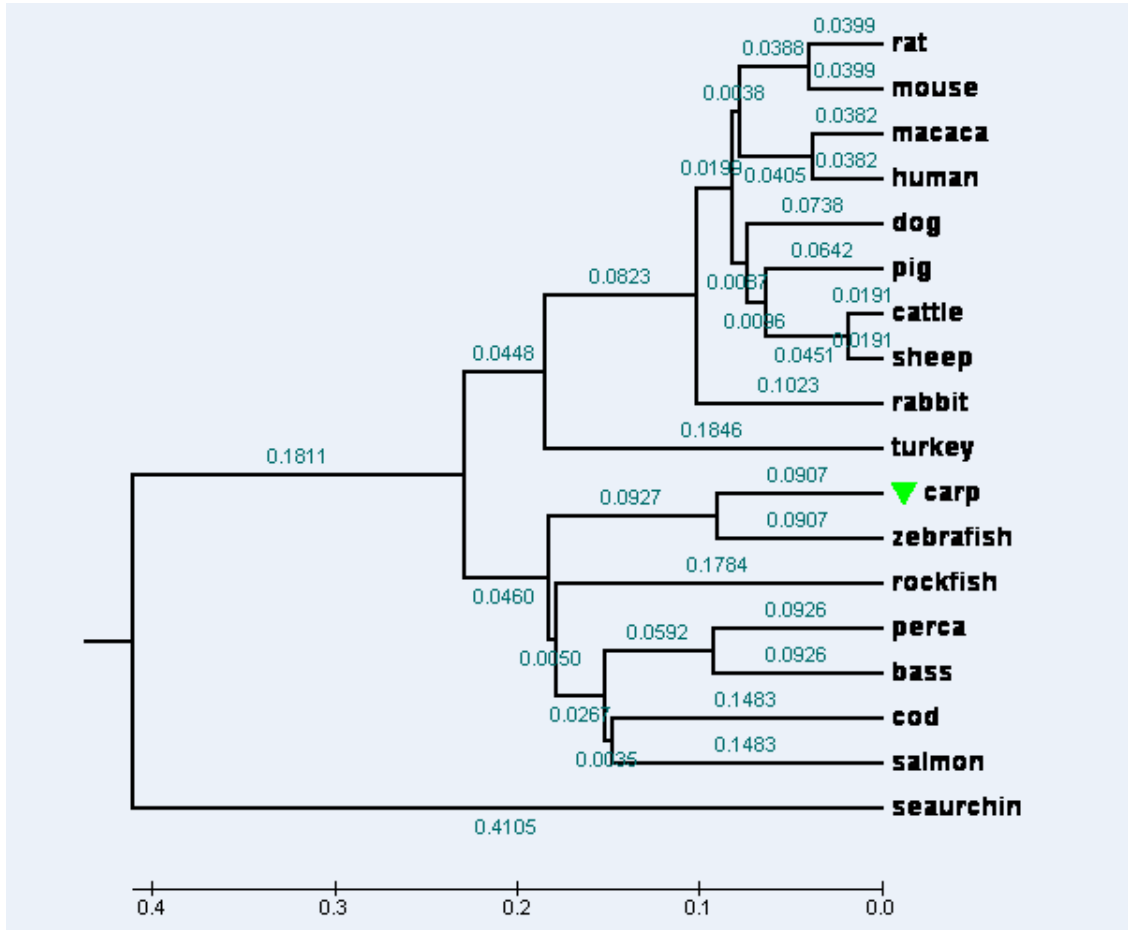

Figure 6. The Phylogenetic relationship of *Cyprinus carpio* L. Pept1 and its orthologues. A molecular phylogenetic tree of Pept1 was generated based on the alignment of the amino acid sequences by MEGA 5.0. The accession numbers for the sequences are as follows: human, *Homo sapiens* (NP_005064.1); *Macaca*, *Macaca fascicularis* (AAQ56235.1); mouse, *Mus musculus* (AAF81666.1); rat, *Rattus norvegicus* (NP_476462.1); cattle, *Bos taurus* (NP_001092848.1); pig, *Sus scrofa* (NP_999512.1); rabbit, *Oryctolagus cuniculus* (NP_001075806.1); turkey， *Meleagris gallopavo* (AAO16604.1); zebra fish, *Danio rerio* (NP_932330.1); rock fish, *Sebastes nebulosus* (ABV82968.1); salmon, *Salmo salar* (BAH24102.1); cod, *Gadus morhua* (AAY17354.1); bass, *Dicentrarchus labrax* (ACI49693.2); *Perca*, *Perca flavescens* (ACX49753.2); dog, *Canis lupus familiaris* (AAL67837.2); sheep, *Ovis aries* (NP_001009758.1); sea urchin, *Strongylocentrotus purpuratusand* (XP_792746.1).

Figure 7. The predicted 3-D structure of Pept1 in intestine of *Cyprinus carpio* L. A, This figure was prepared with PyMOL. TM helixes are shown in colors.

Acknowledgments

We would express our gratitude to Dr Brian. P. Hedlund (associate professor, school of Life Sciences, University of Nevada, Las Vegas) for taking time from his busy schedule to give assistance in writing this paper. This study was supported by Program Science & Technology Innovation Talents in Universities of Henan Province (2010HASTIT020). The Eighth Symposium of World's Chinese Scientists on Nutrition and Feeding of Finfish and Shellfish (Chengdu, China, 2011) provided a very precious opportunity to communicate for this paper, and we appreciate the participants of this symposium put forward some good suggestions for our research.

Conflict of Interest: There is no conflict of interest in respect of this manuscript.

References

[1] Daniel H, Spanier B, Kottra G, Weitz D. (2006) From bacteria to man: archaic proton-dependent peptide transporters at work. Physiology. 21: 93-102.

[2] Dantzig AH, Hoskins JA, Tabas LB, Bright S, Shepard RL, et al. (1994) Association of intestinal peptide transport with a protein related to the cadherin superfamily. Science 264(5157):430-3.

[3] Fei YJ, Kanai Y, Nussberger S, Ganapathy V, Leibach FH, et al. (1994) Expression cloning of a mammalian proton-coupled oligopeptide transporter. Nature 368(6471):563-6.

[4] Perry JR, Basrai MA, Steiner HY, Naider F, Becker JM. (1994) Isolation and characterization of a Saccharomyces cerevisiae peptide transport gene. Mol Cell Biol 14(1):104-15.

[5] Liang R, Fei YJ, Prasad PD, Ramamoorthy S, Han H, et al. (1995) Human Intestinal H^+/peptide cotransporter. Cloning, functional expression, and chromosomal localization. J Biol Chem 270(12):6456-63.

[6] Liu W, Liang R, Ramamoorthy S, Fei YJ, Ganapathy ME, et al. (1995) Molecular cloning of PEPT 2, a new member of the H^+/peptide co-transporter family, from human kidney. Biochim Biophys Acta 1235(2):461-6.

[7] Boll M, Herget M, Wagener M, Weber WM, Markovich D, et al. (1996) Expression cloning and functional characterization of the kidney cortex high-affinity proton-coupled peptide transporter. Proc Natl Acad Sci USA 93(1):284–9.

[8] Ganapathy V, Brandsch M, Leibach FH. (1994) Intestinal transport of amino acids and peptides. In Physiology of the Gastrointestinal Tract (Ed: Johnson LR), pg.1773-94, Raven Press, New York.

[9] Tiruppathi C, Ganapathy V, Leibach FH. (1990) Kinetic evidence for a common transporter for glycylsarcosine and phenylalanylprolylalanine in renal brush-border membrane vesicles. J Biol Chem 265(25):14870–4.

[10] Daniel H, Adibi SA. (1993) Transport of beta-lactam antibiotics in kidney brush border membrane. Determinants of their affinity for the oligopeptide/H^+ symporter. J Clin Invest 92(5):2215–23.

[11] Newstead S, Drew D, Cameron AD, Postis VL, Xia X, et al. (2011) Crystal structure of a prokaryotic homologue of the mammalian oligopeptide–proton symporters, PepT1 and PepT2. EMBO J 30(2):417-26.

[12] Herrera-Ruiz D, Wang Q, Gudmundsson OS, Cook TJ, Smith RL, et al. (2001) Spatial expression patterns of peptide transporters in the human and rat gastrointestinal tracts, Caco-2 in vitro cell culture model, and multiple human tissues. AAPS PharmSci 3(1):E9.

[13] Gbaguidi B, Hakizimana P, Vandenbussche G, Ruysschaert JM. (2007) Conformational changes in a bacterial multidrug transporter are phosphatidylethanolamine-dependent. Cell Mol Life Sci 64(12):1571-82.

[14] Daniel H, Kottra G. (2004) The proton oligopeptide cotransporter family SLC15 in physiology and pharmacology. Pflugers Arch 447(5):610–8.

[15] Covitz KM, Amidon GL, Sadee W. (1998) Membrane topology of the human dipeptide transporter, hPEPT1, determined by epitope insertions. Biochemistry 37(43):15214–21.

[16] Terada T, Saito H, Mukai M, Inui K. (1997) Recognition of beta-lactam antibiotics by rat peptide transporters, PEPT1 and PEPT2, in LLC-PK1 cells. Am J Physiol 273(5Pt2):F706–11.

[17] Doring F, Martini C, Walter J, Daniel H. (2002) Importance of a small N-terminal region in mammalian peptide transporters for substrate affinity and function. J Membr Biol 186(2):55-62.

[18] Biegel A, Knutter I, Hartrodt B, Gebauer S, Theis S, et al. (2006) The renal type H^+/peptide symporter PEPT2: structure-affinity relationships. Amino Acids 31(2):137–56.

[19] Wenzel U, Thwaites DT, Daniel H. (1995) Stereoselective uptake of beta-lactam antibiotics by the intestinal peptide transporter. Br J Pharmacol 116(7):3021-7.

[20] Tamai I, Nakanishi T, Hayashi K, Terao T, Sai Y, et al. (1997) The predominant contribution of oligopeptide transporter PepT1 to intestinal absorption of beta-lactam antibiotics in the rat small intestine. J Pharm Pharmacol 49(8):796-801.

[21] Faria TN, Timoszyk JK, Stouch TR, Vig BS, Landowski CP, et al. (2004) A novel high-throughput pepT1 transporter assay differentiates between substrates and antagonists. Mol Pharm 1(1):67-76.

[22] Ganapathy ME, Huang W, Wang H, Ganapathy V, Leibach FH. (1998) Valacyclovir: a substrate for the intestinal and renal peptide transporters PEPT1 and PEPT2. Biochem Biophys Res Commun 246(2):470-5.

[23] Tsuda M, Terada T, Irie M, Katsura T, Niida A, et al. (2006) Transport characteristics of a novel peptide transporter 1 substrate, antihypotensive drug midodrine, and its amino acid derivatives. J Pharmacol Exp Ther 318(1):455-60.

[24] Chiang CS, Stacey G, Tsay YF. (2004) Mechanisms and functional properties of two peptide transporters, AtPTR2 and fPTR2. J Biol Chem 279(29):30150-7.

[25] Doring F, Dorn D, Bachfischer U, Amasheh S, Herget M, et al. (1996) Functional analysis of a chimeric mammalian peptide transporter derived from the intestinal and renal isoforms. J Physiol 497(Pt3):773-9.

[26] Meredith D, Boyd CA. (1995) Oligopeptide transport by epithelial cells. J Membr Biol 145(1):1-12.

[27] Chen H, Pan Y, Wong EA, Bloomquist JR, Webb KE Jr. (2002) Molecular cloning and functional expression of a chicken intestinal peptide transporter (cPepT1) in Xenopus oocytes and Chinese hamster ovary cells. J Nutr 132(3):387-93.

[28] Yeung AK, Basu SK, Wu SK, Chu C, Okamoto CT, et al. (1998) Molecular identification of a role for tyrosine 167 in the function of the human intestinal proton-coupled dipeptide transporter(hPepT1). Biochem Biophys Commun 250(1):103-7.

[29] Fei YJ, Liu W, Prasad PD, Kekuda R, Oblak TG, et al. (1997) Identification of the histidyl residue obligatory for the catalytic activity of the human H^+/peptide cotransporters PEPT1 and PEPT2. Biochemistry 36(2):452-60.