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Full Length Research Paper

Molecular cloning, sequence analysis and structure prediction of the related to $b^{0,+}$ amino acid transporter (rBAT) in *Cyprinus carpio* L.

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In this study, the full-length cDNA of basic amino acid transporter gene rBAT was cloned from intestinal cells of *Cyprinus carpio* L. using reverse transcription polymerase chain reaction (RT-PCR) and rapid-amplification of cDNA ends (RACE) methods. The amplified product was 2370 bp, including a 42 bp 5'-untranslated region, a 288 bp 3'-untranslated region, and a 2040 bp open reading frame (ORF), which encoded 679 amino acids. The predicted amino acid sequence showed high similarity with that of zebrafish (83.5%), and low similarity with that of rat (50.90%). The 3-D protein models were predicted by the comparative protein modeling program SWISS-MODEL. The prediction result displayed that the *Cyprinus carpio* L. rBAT had a hydrophilic cytoplasmic N terminus, a single membrane-spanning domain, and an extracellular C terminus. The structural core was a β-sheet at the N terminus. The rBAT associates with the light subunit $b^{0,+}$AT by a disulfide bridge with conserved cysteine residues (residues 109). A better understanding of the functional roles and regulation mechanism of rBAT would provide unique opportunities to investigate the biochemical processes underlying amino acid metabolism in *C. carpio* L., and support the foundation for improving aquaculture culture of *C. carpio* L.

Key words: rBAT gene, cDNA sequence analysis, protein tertiary structure, *Cyprinus carpio* L.

INTRODUCTION

Amino acids participate in biosynthetic pathways, act as neurotransmitters, and are essential for metabolic processes. They are mainly transported from the small intestine and the lumina of renal tubules into blood across highly specialized epithelial cells by transporter proteins which are located within the brush border and the basolateral membranes of these epithelial cells (Pickel et al., 1993; Kanai et al., 2003).

Amino acid transports systems can be divided into different categories, based on different criteria. One criterion is substrate specificity, and the other is the Na^+dependency of the rate of transport (Deves and Boyd, 1998). They are classified as neutral amino acid transporters, basic amino acid transporters and acidic amino acid transporters based on substrate selectivity.

Basic amino acid transporter systems include four transport systems:y^+ , $b^{0,+}$, B^{0+} and y^+L (Kanai et al., 2003). System $b^{0,+}$ and system y^+L are members of the heteromeric amino acid transporters which are composed of two subunits: nonglycosylated polytopic membrane proteins (a light subunit), and N-glycosylated type II membrane glycoproteins (a heavy subunit). The two subunits are linked by a disulfide bridge (Dave et al., 2004; Palacin et al., 2005).

The heavy subunits belong to the SLC3 family of amino acid transporters (rBAT and 4F2hc), while the light subunits are members of the SLC7 family of amino acid transporters. The heavy chain rBAT (related to $b^{0,+}$ amino

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Abbreviations: rBAT, Related to $b^{0,+}$ amino acid transporter**;** **$b^{0,+}$AT, $b^{0,+}$** amino acid transporter; **RACE,** rapid-amplification of **cDNA** ends; **ORF,** open reading frame; **SOPMA,** significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments.

Table 1. Sequences of oligonucleotide primers used for PCR and rapid amplification of cDNA ends.

Name	Oligonucleotide sequence (5'→3')	Length (bp)
3' GSP1 rBAT	ATGCCCAAGGAGGTGCTGTTGT	22
3' GSP2 rBAT	GATGTGGAGGACTTCAGGCAGAT	23
3' RACE outer	TACCGTCGTTCCACTAGTGATTT	23
3' RACE inner	CGCGGATCCTCCACTAGTGATTTCACTATAGG	32
5' GSP1 rBAT	GGTGTGGTTTGGTATGAAATCCAT	24
5' GSP2 rBAT	AGGTGACAGTGCCACAATCGTAATG	25
5' RACE outer	CATGGCTACATGCTGACAGCCTA	23
5' RACE inner	CGCGGATCCACAGCCTACTGATGATCAGTCGATG	34

The primers were based on the rBAT sequences of other animals deposited in GenBank.

acid transporter, SLC3A1) associates with the light chain $b^{0,+}$AT ($b^{0,+}$ amino acid transporter, SLC7A9) to form the amino acid transport system $b^{0,+}$ (Fender et al., 2010; Grillo et al., 2008 ;Bartoccioni et al. 2008). System $b^{0,+}$ mediates the absorption and reabsorption of basic amino acids in the small intestine and renal tubules, respectively (Dave et al., 2004). The rBAT protein is expressed in the brush border of the renal epithelial cells of the proximal tubule and the small intestine. The rBAT mRNA is also detected in liver, pancreas and brain (Deves and Boyd,1998). Distinct classes of rBAT mutants cause type I cystinuria which result in increased urinary excretion of cystine and dibasic amino acids and cause cystinuria. Type I cystinuria is a completely recessive disease (Sakamoto et al., 2009). So far, the researches mainly concentrated on the physiological functions and requirements of the basic amino acids in fish and so on (Cheng et al., 2011; Poppi et al., 2011; Zhou et al., 2011), while few studies were reported in absorption mechanism of basic amino acids in *C. carpio* L. on molecular level. In this study, the rBAT gene was first cloned from intestinal cells of *C. carpio* L., and the homology and phylogenetic relation-ship of rBAT genes between *C. carpio* L. and other species were analysed. The secondary structure and tertiary structure of the rBAT protein in *C. carpio* L. was predicted with several computational algorithms.

MATERIALS AND METHODS

Fish acclimation

In this study, *C. carpios* L., with an average body weight of 2.6±0.38 g, grown for 60 days, were used as experimental animals, which were cultured in a 200-L tank filled with dechlorinated water with constant aeration (dissolved oxygen (DO): 6.2±0.2 mg/L, pH 6.5 to 7.5, total ammonia: 2.2 to 2.7 mg/L, average water temperature: 24.5±4.32°C, NO_2^-: 0.03 to 0.07 mg/L, NO_3^-: 1.5 mg/L). The illuminating rhythm was 12 h light and 12 h dark per day. During the period of acclimation, the fishes were fed with commercial pellet feed four times a day (8:30 am, 11:30 am, 14:30 pm and 17:30 pm). After the acclimation, 10 fish were randomly selected and dissected after general anesthesia to obtain the intestines of each fish, and the contents in guts were cleared rapidly. All the operations were conducted under aseptic conditions on ice.

Total RNA extraction and RT-PCR

The prepared guts were quickly frozen immediately in liquid nitrogen and then stored at -80°C. Total RNA was isolated from intestines by the standard TRIzol (purchased from Invitrogen) extraction method. The total RNA (5 mg) was used to synthesize the first-strand cDNA 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 rBAT cDNAs were then amplified by PCR in a total volume of 50 µL, containing 10 mM Tris-HCl (pH9.0), 50 mM KCl, 1.25 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit of Taq polymerase (from Takara, Japan), 5 µL template cDNA and 40 pmol of each primer (Table 1). The following PCR cycling conditions were used as follow: pre-denaturalization at 94°C for 4 min, 35 cycles of denaturation at 94°C for 50 s, annealing at 53 to 55°C for 50 s, extension at 72°C for 1 min 50 s and final extension at 72°C for 10 min. The PCR product was resolved on 1% agarose gels via electrophoresis. Photographs of the gels stained with ethidium bromide are shown in an inverted black/white format (Figure 1).

Cloning and sequencing of rBAT from *C. carpios* L. intestinal cDNA

The amplified bands corresponding to rBAT cDNAs were separated with 1% agarose gel electrophoresis and purified using the Gel Extraction Kit (from Takara, Japan). The purified rBAT cDNAs were combined with the pGEM-T Easy vector (Promega, USA) at 16°C for 8 h, and the ligation mixture was used to transform *Escherichia coli* strain JM109. The plasmid purifications from the overnight-grown colonies were done. For each cDNA, four to six plasmid clones containing rBAT cDNAs were sequenced by ABI3730 using M13+/-universal primers (Takara, Japan).

RESULTS

Isolation of the *C. carpios* L. rBAT cDNA by rapid-amplification of cDNA ends (RACE)

The primers were originally designed from highly con-served regions of rBAT based on the sequence alignment of human, rat, mouse, rabbit, dog, and American opos-sum rBAT cDNA from GenBank. Employing the RACE strategy, the full-length rBAT of *C. carpio* L. was cloned. The 5'-RACE and 3'-RACE results were sequenced and

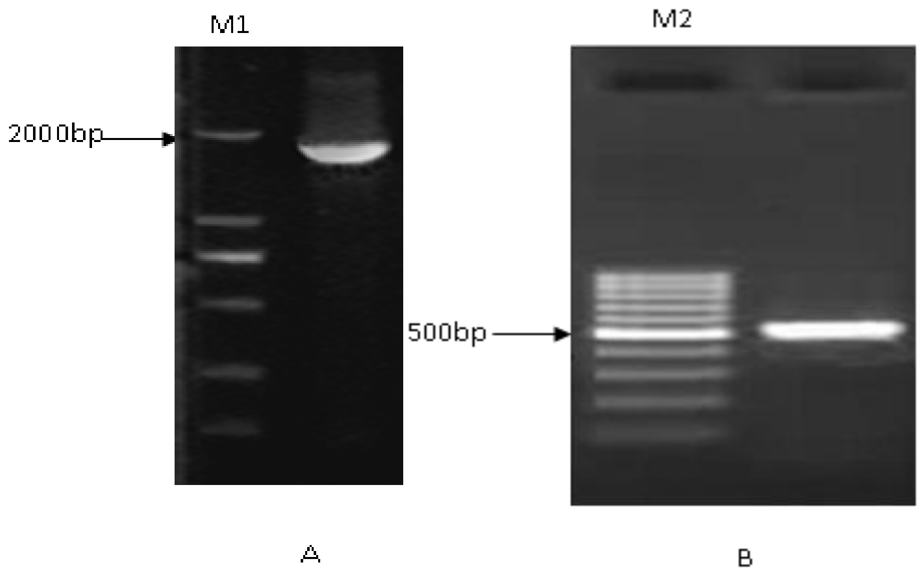

Figure 1. The results of rBAT amplified by RACE-PCR. A, the result of 5′-RACE showing the amplified 1800 bp fragment; B, the result of 3′-RACE showing the amplified 500 bp fragment; M1, 2000 bp DNA ladder; M2, 1000 bp DNA ladder.

spliced to obtain the full-length cDNA (Figure 1). The complete coding sequence of the *C. carpio* L. rBAT cDNA with 2370 nucleotides comprised a coding sequence region with a 2040 bp open reading frame (ORF), a 42 bp 5'-untranslated region and a 288-bp 3'-untranslated region including poly (A).

Sequence analysis of *C. carpios* L. rBAT gene

The deduced amino acid sequence of the *C. carpio* L. rBAT using EXPASY is composed of 679 amino acids with a molecular weight of approximately 78.5 ku and the isoelectric point of 4.96 (Figure 2). The secondary structure of the deduced rBAT amino acid sequence was analyzed to seek potential transmembrane regions using TMHMM Server v. 2.0 (DTU) (Figure 3). One putative transmembrane domain was also identified using significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments (SOPMA) (Figure 4). The rBAT gene contains 28.13% of α-helix, 16.79% of extended strand, 5.60% of β-turn and 49.48% of random coil.

SignalP 3.0 Sercer analysis predicted an N-terminal signal peptide sequence:

(MSSTKITNIDAVELQEGIQNAAFHEDDDDTSNASSSRE QQATSVSVTRPEENEYTQIKPYAGMPKEVLMLYSRKAC YRVPREIIFWLIIACTLALIAMTITIVAL) (Figure 5).

Homology and phylogenetic analysis of rBAT genes

The rBAT cDNA sequence obtained in this study has been submitted to GenBank and assigned the accession number AEX13745.1. The deduced amino acid sequence of *C. carpio* L. rBAT was 69.7, 83.5, 51.4, 50.8, 53.2, 51.9, 51.7, 51.8, 50.7, 53.5 and 51.0% similar to Atlantic salmon (*Salmo salar*), zebrafish (*Danio rerio*), human (*Homo sapiens*), dog (*Canis lupus familiaris*), pig (*Sus scrofa*), rat (*Rattus norvegicus*), mouse (*Mus musculus*), marmoset (*Callithrix jacchus*), rabbit (*Oryctolagus cuniculus*), cattle (*Bos taurus*), and chicken (*Gallus gallus*), respectively. The homology among the sequences was calculated using the Laser-gene analysis software package (Figure 6). The deduced amino acid sequence of rBAT in *C. carpio* L. had the lowest similarity with *Sus scrofa* (50.7%) and the highest similarity with *Danio rerio* (83.5%). The phylogenetic trees were constructed using MEGA5.0. The calculation of the evolutionary trees used the maximum likelihood and nearest-neighbor-interchange (NNI) (Figure 7).

Tertiary structure prediction of rBAT gene

The 3-D protein model in this study was predicted by the comparative protein modeling program SWISS-MODEL automated protein modeling server, based upon deep-sea bacterium *Geobacillus* sp. strain HTA-462 (2ze0A.pdb) Protein Data Bank structure file (Figure 8).

DISCUSSION

The rBAT proteins are members of the expanded solute carrier SLC3A family, and are predominantly expressed in the apical membrane of the intestinal and renal absorptive

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-45   gaaaacaagtgttgttcctagggactgaaggaagcaagaaag
1     atgagttcgaccaaaatcaccaacatcgacgcggtggagctgcaa
1     M  S  S  T  K  I  T  N  I  D  A  V  E  L  Q
46    gaaggcatccagaacgcagcctttcatgaagatgatgatgataca
16    E  G  I  Q  N  A  A  F  H  E  D  D  D  D  T
91    tctaatgcatcaagctctcgagagcagcaggccaccagcgtgtcc
31    S  N  A  S  S  S  R  E  Q  Q  A  T  S  V  S
136   gtcaccaggccggaggagaacgagtacactcagatcaagccgtac
46    V  T  R  P  E  E  N  E  Y  T  Q  I  K  P  Y
181   gctgggatgcccaaagaggtcctgatgttatactccaggaaagcc
61    A  G  M  P  K  E  V  L  M  L  Y  S  R  K  A
226   tgctaccgcgtacctcgagagattattttctggctcatcatcgca
76    C  Y  R  V  P  R  E  I  I  F  W  L  I  I  A
271   tgcaccctggcccttattgccatgaccattacgattgtggcactg
91    C  T  L  A  L  I  A  M  T  I  T  I  V  A  L
316   tcacctcgatgcatgagctggtggcagctgtctccagtctatcag
106   S  P  R  C  M  S  W  W  Q  L  S  P  V  Y  Q
361   gtttatccacgatcattcaaagactcgaatgctgatggtgttgga
121   V  Y  P  R  S  F  K  D  S  N  A  D  G  V  G
406   gatctcaaaggaatcaaggagaaactgagtcattttgagtacctg
136   D  L  K  G  I  K  E  K  L  S  H  F  E  Y  L
451   aacattaaagcagtctggatcagccctttctacaagtctcccatg
151   N  I  K  A  V  W  I  S  P  F  Y  K  S  P  M
496   agagactttggatatgatgtggaggacttcaggcagatcgatcct
166   R  D  F  G  Y  D  V  E  D  F  R  Q  I  D  P
541   atcttcggaaccatggaagactttgacgagctcctggcaagcatg
181   I  F  G  T  M  E  D  F  D  E  L  L  A  S  M
586   catgacaaaggtttaaagctgatcatggattacatcccgaaccac
196   H  D  K  G  L  K  L  I  M  D  Y  I  P  N  H
631   accagcgacaaacacatttggttccaacttagccgtaatggtaca
211   T  S  D  K  H  I  W  F  Q  L  S  R  N  G  T
676   gagccctataaagactactacatctgggttaactgcacacgagac
226   E  P  Y  K  D  Y  Y  I  W  V  N  C  T  R  D
721   aagcctccaaacaactgggtgagtgtcttcgggaattccacctgg
241   K  P  P  N  N  W  V  S  V  F  G  N  S  T  W
766   gagtatgatgaggtacgacaacagtgctatttccatcagttcctg
256   E  Y  D  E  V  R  Q  Q  C  Y  F  H  Q  F  L
811   aaggaacagcctgacctgaactaccgtaaccctcgagtcatagag
271   K  E  Q  P  D  L  N  Y  R  N  P  R  V  I  E
856   gagatgacggacataatccatttctggctgaagaagggggtggat
286   E  M  T  D  I  I  H  F  W  L  K  K  G  V  D
901   gggttccgcatggacgctgtgaaacacatgcttgaggccacacat
301   G  F  R  M  D  A  V  K  H  M  L  E  A  T  H
946   ttgagaaatgaaccccaggtcgaccctgaccaagatccatcgact
316   L  R  N  E  P  Q  V  D  P  D  Q  D  P  S  T
991   gtggacacagagtttgagctgttccatgactacacctacacacaa
331   V  D  T  E  F  E  L  F  H  D  Y  T  Y  T  Q
1036  cagggcttacatgagattctgacaaactggaggatagatctggac
346   Q  G  L  H  E  I  L  T  N  W  R  I  D  L  D
1081  gcctacagcagagagcccggccgctacaggttcatggtgatagag
361   A  Y  S  R  E  P  G  R  Y  R  F  M  V  I  E
1126  tgttatgattatgaagaaatagataaaaccatgaggtactatggc
376   C  Y  D  Y  E  E  I  D  K  T  M  R  Y  Y  G
1171  acgagctatgtcactgaaagcgacttcccctttaacttctatctc
391   T  S  Y  V  T  E  S  D  F  P  F  N  F  Y  L
1216  ttgtaccttcctgatgatctgtcaggaaatcaagccaaaagcttg
406   L  Y  L  P  D  D  L  S  G  N  Q  A  K  S  L
1261  gttcatttatggatgtcaaacatgccgaagggaaaatggccaaac
421   V  H  L  W  M  S  N  M  P  K  G  K  W  P  N
1306  tgggtggtgggaaaccatgacaagccacgtataggctcaagtgct
436   W  V  V  G  N  H  D  K  P  R  I  G  S  S  A
1351  ggtacagaatatatacgtgctataaacatgctgttgttaacgctg
451   G  T  E  Y  I  R  A  I  N  M  L  L  L  T  L
1396  cctggaactcctacaacatactatggagaagagattggcatggtg
466   P  G  T  P  T  T  Y  Y  G  E  E  I  G  M  V
1441  aacgtaaatgtatctgtaattcaggatccttttggacagcatgat
481   N  V  N  V  S  V  I  Q  D  P  F  G  Q  H  D
1486  ccaagcaacagtcgggacccgcagcgaacaccaatgcagtgggac
496   P  S  N  S  R  D  P  Q  R  T  P  M  Q  W  D
1531  gataagctcaatgctggttttagtgacagtgaaaatggcacatgg
511   D  K  L  N  A  G  F  S  D  S  E  N  G  T  W
1576  ctagatatcgctccagactacagcactgtcaatgtagagcttcag
526   L  D  I  A  P  D  Y  S  T  V  N  V  E  L  Q
1621  caggctgatgcacactccaccgtttcacagtatcgtgctctgagt
541   Q  A  D  A  H  S  T  V  S  Q  Y  R  A  L  S
1666  ttgctccgaggggctgagttggcactgtcccgaggctggttctgc
556   L  L  R  G  A  E  L  A  L  S  R  G  W  F  C
1711  ttcgtctggagcgatgtcaacgtatttgcttatttgcgtgagctg
571   F  V  W  S  D  V  N  V  F  A  Y  L  R  E  L
1756  gatgggctcaacaaagccttcctggtggttctaaacttcggcgag
586   D  G  L  N  K  A  F  L  V  V  L  N  F  G  E
1801  gacactacaacagacttgtcttcagttactgagttgccagatact
601   D  T  T  T  D  L  S  S  V  T  E  L  P  D  T
1846  ctcactgtgcatttaagcacagtgccaataagtcaaaagactttc
616   L  T  V  H  L  S  T  V  P  I  S  Q  K  T  F
1891  actaaatccagaattccaacatctcgagggcaaggaatgctccta
631   T  K  S  R  I  P  T  S  R  G  Q  G  M  L  L
1936  gaatattccaccaatcagcgctttcacctcaaccatgcatctgag
646   E  Y  S  T  N  Q  R  F  H  L  N  H  A  S  E
1981  tgctctgtttctgagaaagcctgctacttgcctgcactggatatt
661   C  S  V  S  E  K  A  C  Y  L  P  A  L  D  I
2026  ctgtacaagtgttgaagatagaagcgacacagtgacaaacgataa
676   L  Y  K  C  *
2071  aggctatctattccttgttttatttcggtggtgtcatgctggcta
2116  gtcccgcccactttgaaatctcattggtccaaaatctcactcgat
2161  attattgtgatagtgtgtatcgtttgtgttcagtgtaaactggca
2206  agttgcctggcgatgcaaactgtttttgtcagattaacttttatt
2251  caaaaggaactagcatctatgtaacaaaataaatgtttggtttgt
2296  taagaaaaatctgaaaaaaaaaaaaaaaaaaaa
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Figure 2. Nucleotide and deduced amino acid sequence of rBAT in the intestine of *Cyprinus carpio* L. The sequence contains a single open reading frame which encodes a protein with 679 amino acids. The complete 5'-untranslated region was 42 nucleotides.

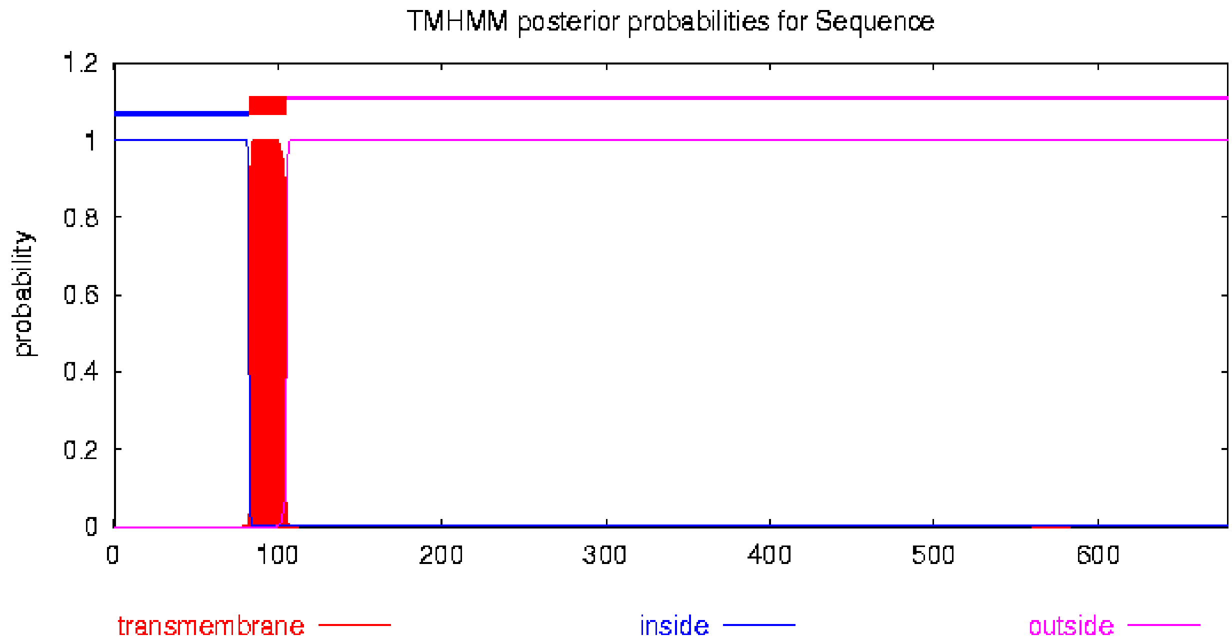


Figure 3. Secondary structure model and the predicted transmembrane domain of rBAT predicted by TMHMM Server v. 2.0. The C-terminal domain of rBAT is predicted to extend from the cell membrane, and the N-terminal domain of rBAT is in the cytoplasm.

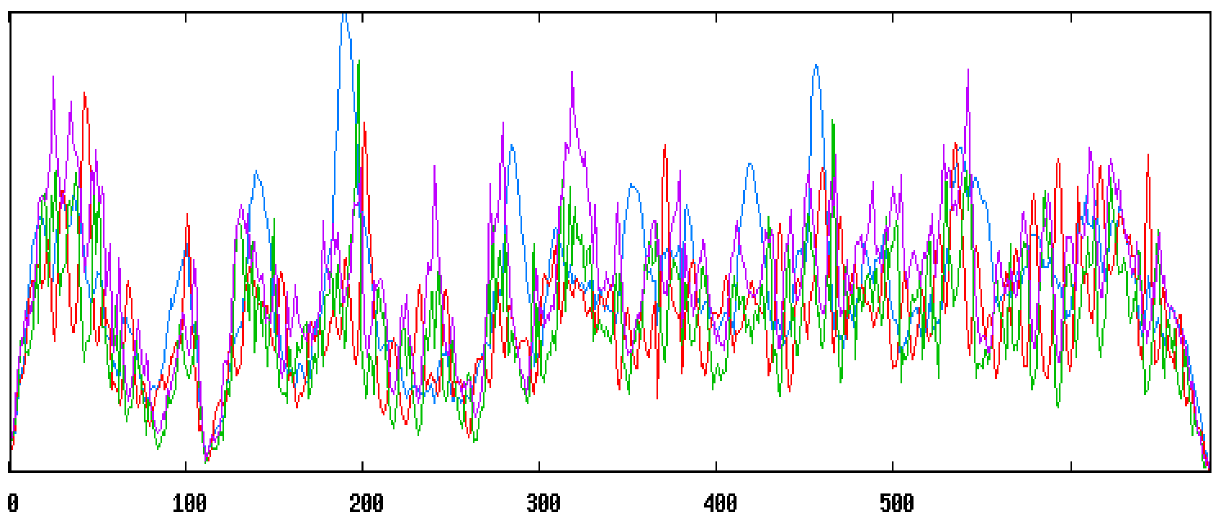


Figure 4. SOPMA result for rBAT from *Cyprinus carpio* L. intestine. The rBAT gene contains 28.13% of α-helix, 16.79% of extended strand, 5.60% of β-turn and 49.48% of random coil. SOPMA, significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments.

epithelial cells. As a member of the basic amino acid transporter family, rBAT plays an important role in the absorption and reabsorption of cystine and basic amino acids such as lysine, arginine and ornithine (Ganapathy 2009). In this study, rBAT was first cloned and characterized in *C. carpio* L., and subsequently used to obtain new insights into the molecular mechanism of the $b^{0,+}$ system. This study performed a foundation for delineating the molecular evolution and nutritional characteristics between C. *carpio* L. and other fish strains or mammals.

The rBAT subunit from *C. carpio* L. showed high similarity with zebrafish and salmon, as compared with mammalian and avian rBAT homology. The nucleotide sequences of rBAT gene from several species were classified into two major groups. The rBAT subunits of mammals and chicken were clustered into one group.

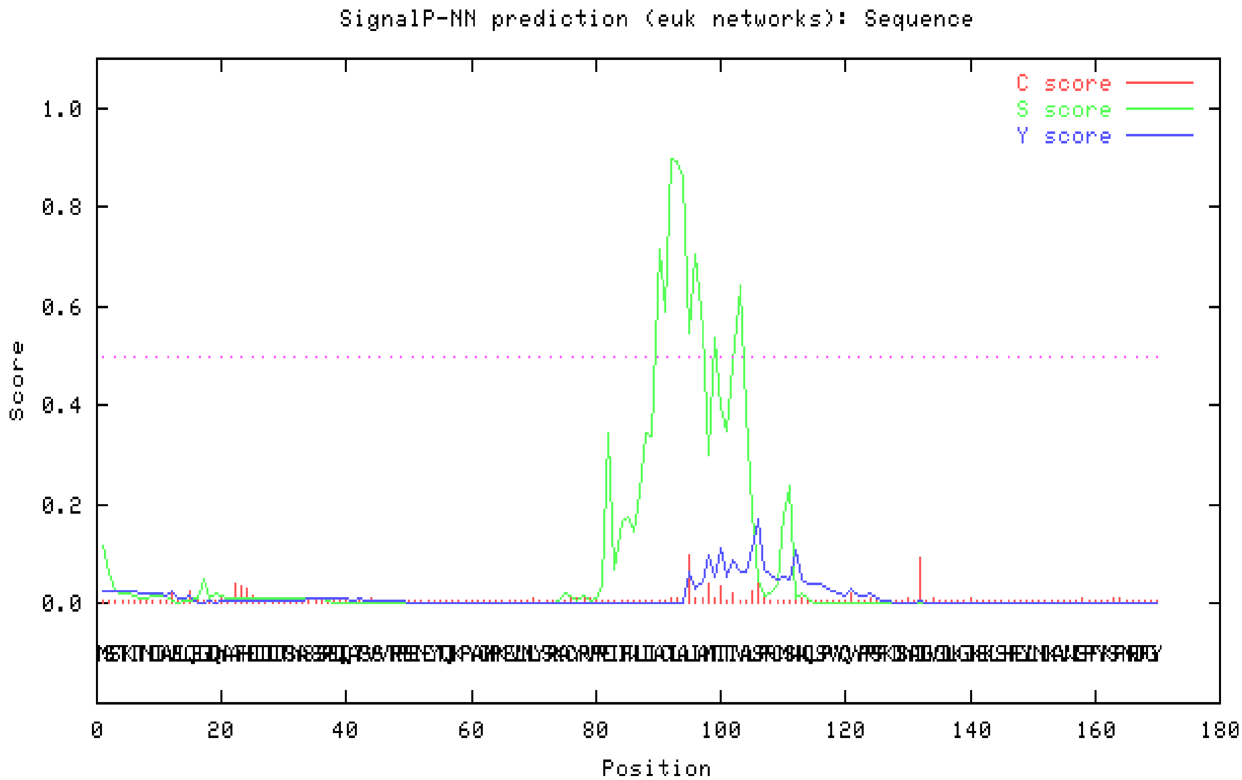


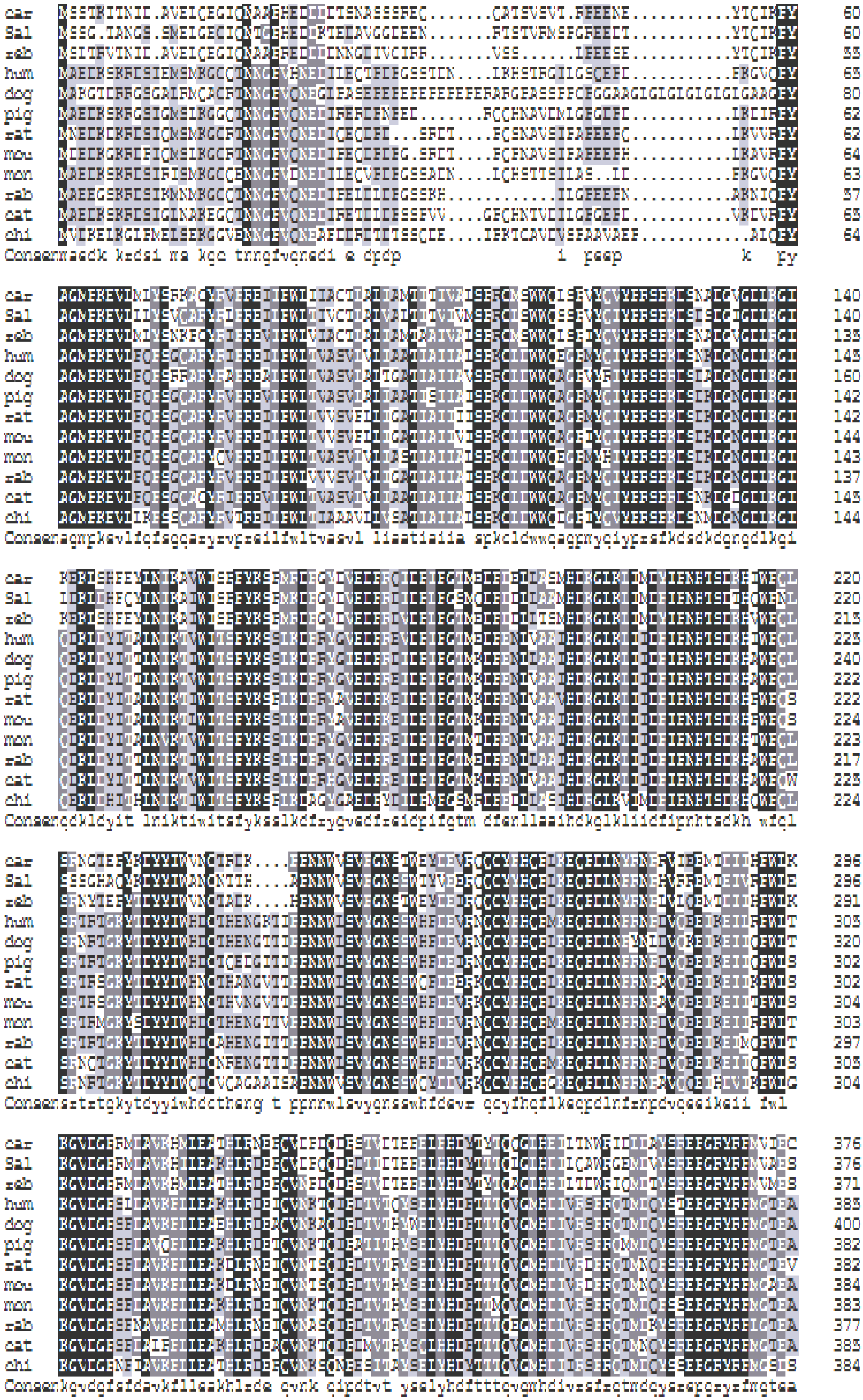
Figure 5. SignalP 3.0 Sercer analysis predicted a signal peptide of carp rBAT positioned in the amino-terminal (N-terminal) sequence (MSSTKITNIDAVELQEGIQNAAFHEDDDDTSNASSSREQQATSVSVTRPEENEYTQIKPYAGMPKEVLMLYSRKACYRVPREIIFWLIIACTLALIAMTITIVAL).

The rBAT subunit of *C. carpio L.*, zebrafish and salmon were clustered into another group. The homology of rBAT was the highest between *C. carpio* L. and zebrafish (Figure 7). The results of comparative sequence analysis showed that the homologous sequences of fishes shared more than 69% amino acid similarity and those from fishes, mammals, and birds shared more than 50% amino acid similarity. Totally, based on the characteristic analysis of the transmembrane and cytoplasmic domains, it was indicated to be the higher conservative between *C. carpio* L. and other species.

The rBAT gene encoded a predicted glycoprotein with a hydrophilic cytoplasmic domain, a single membrane-spanning domain, and a bulky extracellular domain. An internal hydrophobic region (residues 83 to 105) was predicted to be membrane spanning. This structure was unlike other transporters and membrane channels (Newstead et al., 2011), most of them had multiple membrane-spanning regions. Its bulky extracellular domains showed significant homology with the family of α-amylases and α-glucosidases (Gabrisko and Janecek, 2009), which consisted of three domains; the N-terminal, subdomain, and the C-terminal domains. The rBAT subunit from *C. carpio* L. contained three catalytic residues (aspartate: 213 and 289; glutamate: 375, 475 and 476; histidine: 215) of the α-amylases family. This may mean that the eventuality of α-glucosidase activity of true rBATs cannot be unambiguously eliminated. The core of the transporter had seven parallel β-sheets, which form a β barrel at the N-terminal (residues 110 to 212, 280 to 315, and 339 to 581). The subdomain (residues 213 to 279 and 316 to 338) was inserted into the N-terminal domain. The C-terminal domain (residues 582 to 661) followed the N-terminal and subdomains. The rBAT associates the light subunit $b^{0,+}$AT with a disulfide bridge including conserved cysteine residues (residues 109).

Conclusion

In this study, we obtained the full-length rBAT gene sequence (2370 bp) of *C. carpio* L. Homology analysis and phylogenetic relationship demonstrate that rBAT gene of *C. carpio* L. displayed the highest similarity with *Danio rerio* (83.5%) and lowest similarity with the rat (50.90%). The 3-D protein models for rBAT protein were predicted and the predicting result indicate that rBAT protein of *C. carpio* L. only had a single membrane-spanning domain. This study would refer to further studies on protein expression and specific antibody



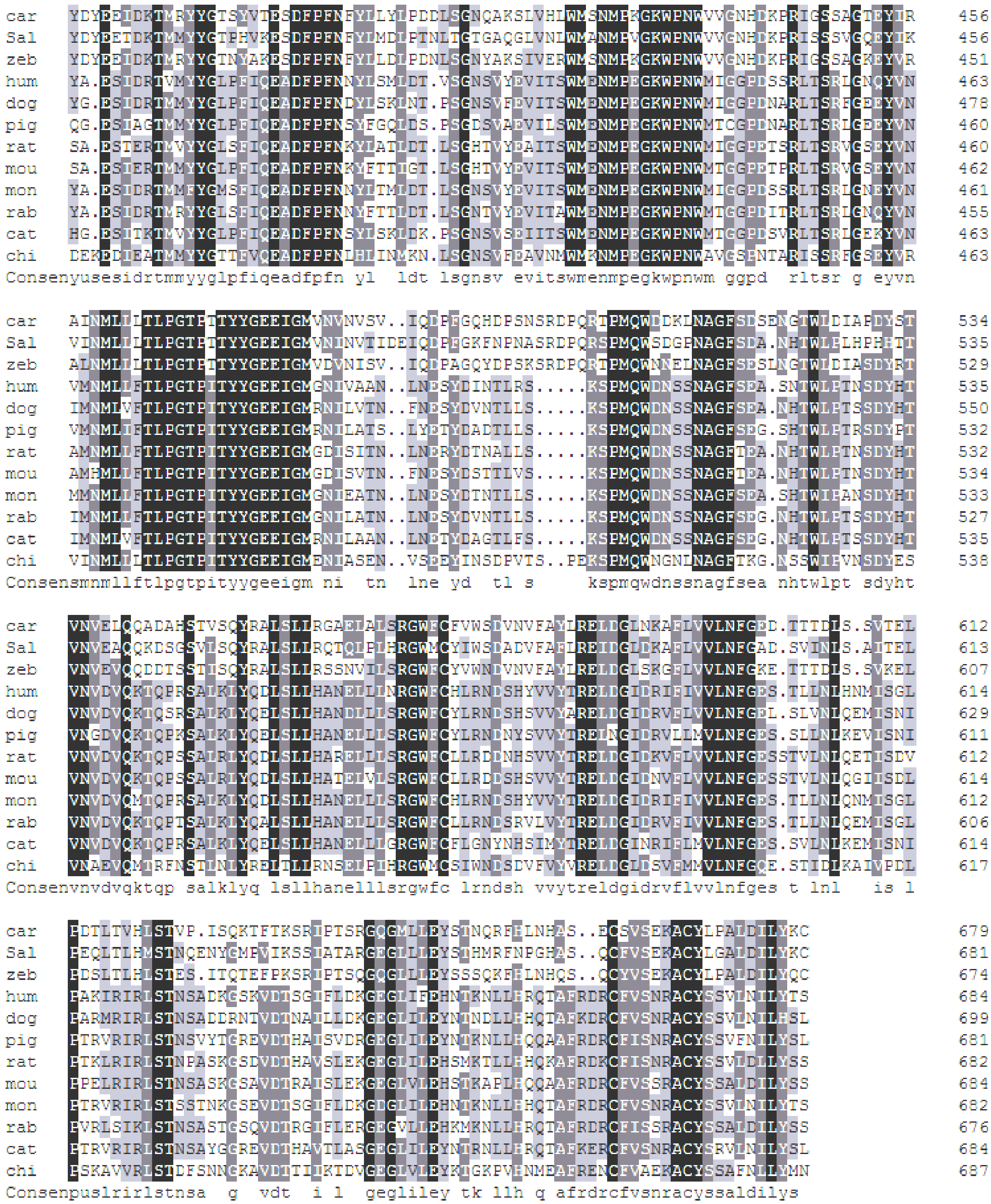


Figure 6. Alignment of deduced amino acid sequences of the rBAT subunit from the *Cyprinus carpio* L. (car), *Salmo salar* (sal), zebrafish (zeb), human (hum), dog (dog), pig (pig), rat (rat), mouse (mou), monkey (mon), rabbit (rab), cattle (cat) and chicken (chi). These protein sequences were aligned using the Clustal program. 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.

preparation and also support further research on the absorption mechanisms of basic amino acids in *C. carpio* L. on molecular level.

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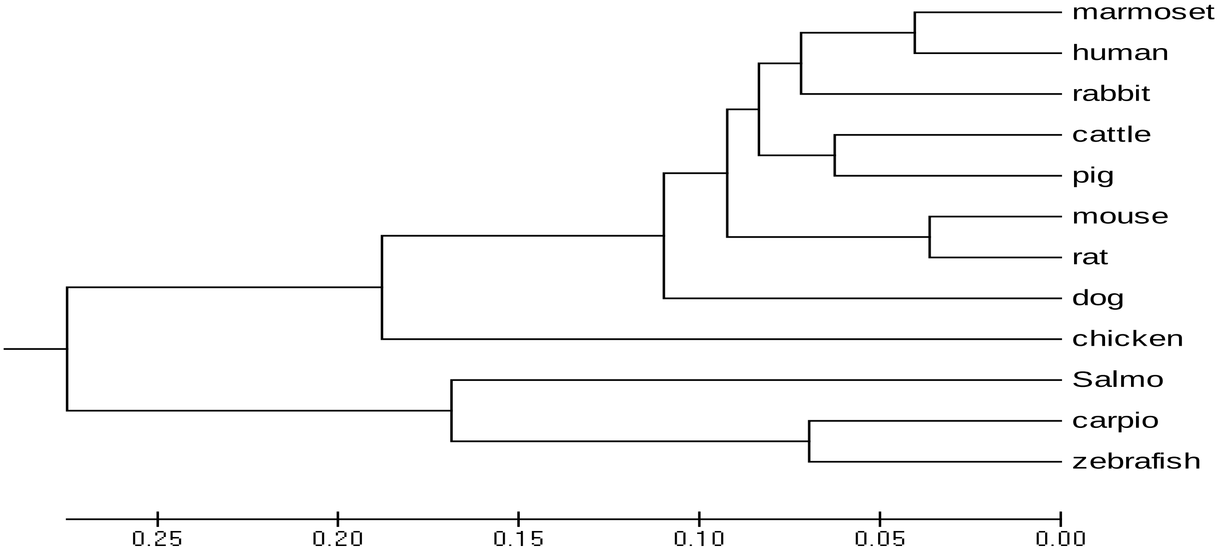

Figure 7. The phylogenetic relationship of fish rBAT and its orthologues. A molecular phylogenetic tree of rBAT was generated based on the alignment of the amino acid sequences by MEGA5. The accession numbers for the sequences are as follows: human, *Homo sapiens* (AAB39829); mouse, *Mus musculus* (AAH13441); rat, *Rattus norvegicus* (NP_058912); rabbit, *Oryctolagus cuniculus* (AAA31391); marmoset, *Callithrix jacchus* (XP_002757851); dog, *Canis lupus familiaris* (AAG34759); cattle, *Bos taurus* (NP_001029805); pig, *Sus scrofa* (ACB46191); chicken, *Gallus gallus* (XP_426125); salom, *Salmo salar* (ACN11390); zebrafish, *Danio rerio* (XP_685969).

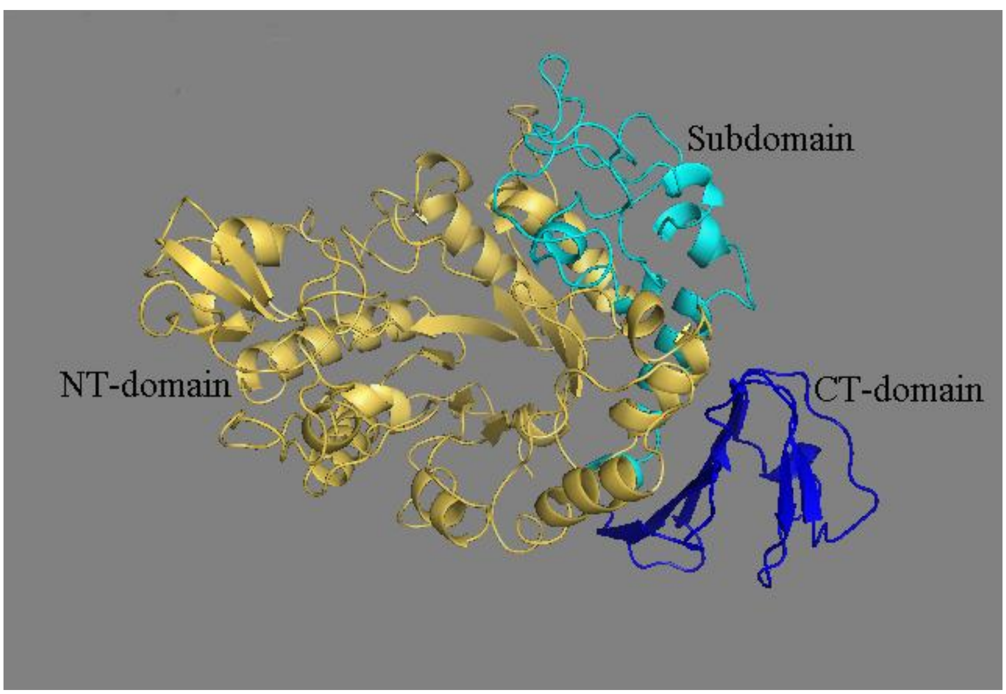

Figure 8. The predicted 3-D structure of rBAT in intestine of Cyprinus carpio L. A, The N-terminal, subdomain, and C-terminal domains are shown in yellow, cyan, and blue, respectively. This figure was prepared with PyMOL.

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