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Molecular cloning and nucleotide sequence analysis of mRNA for human kidney ornithine aminotransferase

An examination of ornithine aminotransferase isozymes between liver and kidney

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The cDNA encoding ornithine aminotransferase (EC 2.6.1.13; OAT) was isolated from a human kidney cDNA library. The isolated cDNA contained the entire protein coding region and partial 3'- and 5'-untranslated regions. The nucleotide sequences of human kidney OAT cDNA were absolutely homologous with those of human liver OAT cDNA, and human kidney and liver OAT fused completely against the antibody to human kidney OAT in an Ouchterlony double diffusion test. These findings settled the controversy as to which characteristics of liver and kidney OAT isozymes are different. An N-terminal sequence analysis of purified mature human kidney OAT clarified that the leader peptide was cleaved between Gln-35 and Gly-36.

Ornithine aminotransferase; cDNA; Nucleotide sequence; Pyridoxal phosphate binding site; Isozyme; (Human liver, Human kidney)

1. INTRODUCTION

Ornithine aminotransferase (OAT) (L-ornithine:2-oxo-acid aminotransferase, EC 2.6.1.13) is a mitochondrial matrix enzyme which catalyzes a reversible reaction of interconversion between ornithine and glutamic γ-semialdehyde (cyclized form, Δ^1-pyrroline-5-carboxylate), and the latter product can be reversibly converted to glutamate or proline. This enzyme is existent in many tissues, including liver, kidney, small intestine, brain and eye. Of these, liver and kidney OAT differ significantly in their regulation by various hormonal and nutritional factors. In order to account for this difference, Volpe et al. [1] postulated the presence of two organ-specific enzymes, namely, liver OAT, which is involved in the synthesis of ornithine for urea formation, and kidney OAT, which participates in ornithine degradation to glutamate or proline. The same authors [2] also clarified that the two isozymes were synthesized at different specific stages of the HeLa cell cycle. In addition, Kalita et al. [3] reported that rat liver and kidney OATs exhibited distinct physicochemical properties which differed in heat lability and cysteine contents. On the other hand, Sanada et al. [4] and Yip and Collins [5] concluded that the two enzymes were not distinguishable by chemical or physical methods. Recently, the cDNA for OAT mRNA has been cloned from human liver [6] and retinoblastoma cell lines [7]. However, controversial studies on the different characteristics of liver and kidney isozymes have not been settled so far [1–5].

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Here we report how we independently isolated the cDNA for OAT mRNA from a human kidney cDNA library and determined nucleotide sequences in an attempt to settle this historical controversy.

2. MATERIALS AND METHODS

2.1. *Preparation of rat liver OAT cDNA*

The rat OAT cDNA was isolated from a rat liver cDNA library constructed in plasmid pBR322, using a 33mer oligonucleotide probe, involving the rat liver OAT active site sequences previously reported [8]. A cDNA fragment of about 250 bp digested with *Bam*HI and *Pst*I (r1OAT-cDNA 250) was used as a probe.

2.2. *Screening of the human kidney OAT cDNA*

A human kidney cDNA library constructed in phage λgt10 (Clontec Laboratories, Inc., Palo Alto, CA) was screened by the procedure of Benton and Davis [9], using r1OAT-cDNA 250, which was labeled by random oligonucleotide priming [10].

2.3. *DNA sequence analysis*

The hkOAT-cDNA clones (see section 3.1) were digested with appropriate restriction enzymes and the resulting fragments were subcloned into the M13 mp18 and mp19 vectors. The cDNA inserts were sequenced by the dideoxy chain termination method [11].

3. RESULTS AND DISCUSSION

3.1. *Isolation and characterization of human kidney OAT cDNA*

Using the r1OAT-cDNA 250 as a probe, eleven positive plaques were isolated from 400000 clones by screening of a human kidney cDNA library. The positive clones were divided into two groups containing cDNA inserts of approximately 1.1 and 1.5 kbp, respectively (hkOAT-cDNAs). A partial restriction map of the 1.5 kbp hkOAT-cDNA and the DNA sequence strategy are shown in fig.1. Fig.2 presents the nucleotide sequence of the 1.5 and 1.1 kbp hkOAT-cDNAs. The 1.5 kbp hkOAT-cDNA contains the entire protein coding region of 1317 nucleotides, specifying a protein of 48479 Da molecular mass, but lacks the partial 5′- and 3′-untranslated regions compared with human liver OAT cDNA [6]. The 1.1 kbp hkOAT-cDNA lacks the entire 5′-untranslated region, but contains the partial protein coding region and the partial 3′-untranslated region. Nucleotide sequence analysis showed that the nucleotide sequence of the 5′-untranslated region, the protein coding region

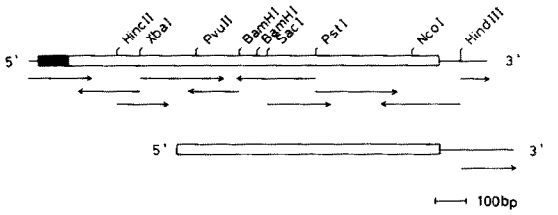

Fig.1. Partial restriction map and sequence strategy of human kidney OAT cDNA. The sequence strategy is summarized beneath the map with horizontal arrows indicating the direction and extent of each sequence determination. The boxed region represents the open reading frame, and the black region shows the mitochondrial signal sequence. The thin bars indicate 5′- and 3′-untranslated regions.

and the 3′-untranslated region are absolutely homologous with human liver OAT cDNA [6]. Furthermore, on an Ouchterlony double diffusion plate, the precipitation lines of the specific antibody IgG for kidney OAT fused completely with liver, kidney and brain OAT (fig.3). These findings present critical evidence that liver and kidney enzymes do not constitute one isozyme, thereby settling the previously mentioned controversy [1–5]. We therefore propose that differential regulation of the mRNA level of OAT in kidney and liver and, if any, in the cell cycle of HeLa cells may be responsible for the 5′-upstream region of the OAT gene. Furthermore, elucidation of the regulation of ornithine metabolism would be more facilitated by investigating the related genes, which are those of the enzymes of ornithine synthetic and degradative pathways: Δ^1-pyrroline-5-carboxylate synthetase, proline dehydrogenase, ornithine carbamoyltransferase, and arginine amidohydrase, Δ^1-pyrroline-5-carboxylate reductase, Δ^1-pyrroline-5-carboxylate dehydrogenase.

3.2. *Mitochondrial signal sequence*

Most nuclear-encoded mitochondrial proteins are synthesized as larger precursors with N-terminal leader peptides. The sequence of the N-terminal 5 residues of the purified mature human kidney OAT subunit was determined by a gas phase protein sequencer (Model 470A, Applied Biosystems, CA, USA). Our sequence data revealed that the leader peptide of human kidney OAT was cleaved after Gln-35 to leave an N-terminal Gly-36. Furthermore, mature rat liver OAT was also processed after Gln-35 (Oyama, R. and

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5'   Kidney         GAATTCGTCAGATCTGTGGTTTTTCTACTTGAAGGACACA   -1
     Liver      GAATTCCGCTGTCAGATCTGTGGTTTTTCTACTTGAAGGACACA   -1

Met-Phe-Ser-Lys-Leu-Ala-His-Leu-Gln-Arg-Phe-Ala-Val-Leu-Ser-Arg-Gly-Val-His-Ser-Ser-Val-Ala-Ser-Ala-Thr-Ser-Val-Ala-Thr   30
ATG TTT TCC AAA CTA GCA CAT TTG CAG AGG TTT GCT GTA CTT AGT CGC GGA GTT CAT TCT TCA GTG GCT TCT GCT ACA TCT GTT GCA ACT   90
ATG TTT TCC AAA CTA GCA CAT TTG CAG AGG TTT GCT GTA CTT AGT CGC GGA GTT CAT TCT TCA GTG GCT TCT GCT ACA TCT GTT GCA ACT   90

                   |
Lys-Lys-Thr-Val-Gln|Gly-Pro-Pro-Thr-Ser-Asp-Asp-Ile-Phe-Glu-Arg-Glu-Tyr-Lys-Tyr-Gly-Ala-His-Asn-Tyr-His-Pro-Leu-Pro-Val   60
AAA AAA ACA GTC CAA|GGC CCT CCA ACC TCT GAT GAC ATT TTT GAA AGG GAA TAT AAG TAT GGT GCA CAC AAC TAC CAT CCT TTA CCT GTA   180
AAA AAA ACA GTC CAA GGC CCT CCA ACC TCT GAT GAC ATT TTT GAA AGG GAA TAT AAG TAT GGT GCA CAC AAC TAC CAT CCT TTA CCT GTA   180

Ala-Leu-Glu-Arg-Gly-Lys-Gly-Ile-Tyr-Leu-Trp-Asp-Val-Glu-Gly-Arg-Lys-Tyr-Phe-Asp-Phe-Leu-Ser-Ser-Tyr-Ser-Ala-Val-Asn-Gln   90
GCC CTG GAG AGA GGA AAA GGT ATT TAC TTA TGG GAT GTA GAA GGC AGA AAA TAT TTT GAC TTC CTG AGT TCT TAC AGT GCT GTC AAC CAA   270
GCC CTG GAG AGA GGA AAA GGT ATT TAC TTA TGG GAT GTA GAA GGC AGA AAA TAT TTT GAC TTC CTG AGT TCT TAC AGT GCT GTC AAC CAA   270

Gly-His-Cys-His-Pro-Lys-Ile-Val-Asn-Ala-Leu-Lys-Ser-Gln-Val-Asp-Lys-Leu-Thr-Leu-Thr-Ser-Arg-Ala-Phe-Tyr-Asn-Asn-Val-Leu   120
GGG CAT TGT CAC CCC AAG ATT GTG AAT GCT CTG AAG AGT CAA GTG GAC AAA TTG ACC TTA ACA TCT AGA GCT TTC TAT AAT AAC GTA CTT   360
GGG CAT TGT CAC CCC AAG ATT GTG AAT GCT CTG AAG AGT CAA GTG GAC AAA TTG ACC TTA ACA TCT AGA GCT TTC TAT AAT AAC GTA CTT   360

Gly-Glu-Tyr-Glu-Glu-Tyr-Ile-Thr-Lys-Leu-Phe-Asn-Tyr-His-Lys-Val-Leu-Pro-Met-Asn-Thr-Gly-Val-Glu-Ala-Gly-Glu-Thr-Ala-Cys   150
GGT GAA TAT GAG GAG TAT ATT ACT AAA CTT TTC AAC TAC CAC AAA GTT CTT CCT ATG AAT ACA GGA GTG GAG GCT GGA GAG ACT GCC TGT   450
GGT GAA TAT GAG GAG TAT ATT ACT AAA CTT TTC AAC TAC CAC AAA GTT CTT CCT ATG AAT ACA GGA GTG GAG GCT GGA GAG ACT GCC TGT   450

Lys-Leu-Ala-Arg-Lys-Trp-Gly-Tyr-Thr-Val-Lys-Gly-Ile-Gln-Lys-Tyr-Lys-Ala-Lys-Ile-Val-Phe-Ala-Ala-Gly-Asn-Phe-Trp-Gly-Arg   180
AAA CTA GCT CGT AAG TGG GGC TAT ACC GTG AAG GGC ATT CAG AAA TAC AAA GCA AAG ATT GTT TTT GCA GCT GGG AAC TTC TGG GGT AGG   540
AAA CTA GCT CGT AAG TGG GGC TAT ACC GTG AAG GGC ATT CAG AAA TAC AAA GCA AAG ATT GTT TTT GCA GCT GGG AAC TTC TGG GGT AGG   540

Thr-Leu-Ser-Ala-Ile-Ser-Ser-Ser-Thr-Asp-Pro-Thr-Ser-Tyr-Asp-Gly-Phe-Gly-Pro-Phe-Met-Pro-Gly-Phe-Asp-Ile-Ile-Pro-Tyr-Asn   210
ACG TTG TCT GCT ATC TCC AGT TCC ACA GAC CCA ACC AGT TAC GAT GGT TTT GGA CCA TTT ATG CCG GGA TTC GAC ATC ATT CCC TAT AAT   630
ACG TTG TCT GCT ATC TCC AGT TCC ACA GAC CCA ACC AGT TAC GAT GGT TTT GGA CCA TTT ATG CCG GGA TTC GAC ATC ATT CCC TAT AAT   630

Asp-Leu-Pro-Ala-Leu-Glu-Arg-Ala-Leu-Gln-Asp-Pro-Asn-Val-Ala-Ala-Phe-Met-Val-Glu-Pro-Ile-Gln-Gly-Glu-Ala-Gly-Val-Val-Val   240
GAT CTG CCC GCA CTG GAG CGT GCT CTT CAG GAT CCA AAT GTG GCT GCG TTC ATG GTA GAA CCA ATT CAG GGT GAA GCA GGC GTT GTT GTT   720
GAT CTG CCC GCA CTG GAG CGT GCT CTT CAG GAT CCA AAT GTG GCT GCG TTC ATG GTA GAA CCA ATT CAG GGT GAA GCA GGC GTT GTT GTT   720

Pro-Asp-Pro-Gly-Tyr-Leu-Met-Gly-Val-Arg-Glu-Leu-Cys-Thr-Arg-His-Gln-Val-Leu-Phe-Ile-Ala-Asp-Glu-Ile-Gln-Thr-Gly-Leu-Ala   270
CCG GAT CCA GGT TAC CTA ATG GGA GTG CGA GAG CTC TGC ACC AGG CAC CAG GTT CTC TTT ATT GCT GAT GAA ATA CAG ACA GGA TTG GCC   810
CCG GAT CCA GGT TAC CTA ATG GGA GTG CGA GAG CTC TGC ACC AGG CAC CAG GTT CTC TTT ATT GCT GAT GAA ATA CAG ACA GGA TTG GCC   810

Arg-Thr-Gly-Arg-Trp-Leu-Ala-Val-Asp-Tyr-Glu-Asn-Val-Arg-Pro-Asp-Ile-Val-Leu-Leu-Gly-Lys-Ala-Leu-Ser-Gly-Gly-Leu-Tyr-Pro   300
AGA ACT GGT AGA TGG CTG GCT GTT GAT TAT GAA AAT GTC AGA CCT GAT ATA GTC CTC CTT GGA AAG GCC CTT TCT GGG GGC TTA TAC CCT   900
AGA ACT GGT AGA TGG CTG GCT GTT GAT TAT GAA AAT GTC AGA CCT GAT ATA GTC CTC CTT GGA AAG GCC CTT TCT GGG GGC TTA TAC CCT   900

Val-Ser-Ala-Val-Leu-Cys-Asp-Asp-Asp-Ile-Met-Leu-Thr-Ile-Lys-Pro-Gly-Glu-His-Gly-Ser-Thr-Tyr-Gly-Gly-Asn-Pro-Leu-Gly-Cys   330
GTG TCT GCA GTG CTG TGT GAT GAT GAC ATC ATG CTG ACC ATT AAG CCA GGG GAG CAT GGG TCC ACA TAC GGT GGC AAT CCA CTA GGC TGC   990
GTG TCT GCA GTG CTG TGT GAT GAT GAC ATC ATG CTG ACC ATT AAG CCA GGG GAG CAT GGG TCC ACA TAC GGT GGC AAT CCA CTA GGC TGC   990

Arg-Val-Ala-Ile-Ala-Ala-Leu-Glu-Val-Leu-Glu-Glu-Glu-Asn-Leu-Ala-Glu-Asn-Ala-Asp-Lys-Leu-Gly-Ile-Ile-Leu-Arg-Asn-Glu-Leu   360
CGA GTG GCC ATC GCA GCC CTT GAG GTT TTA GAA GAA GAA AAC CTT GCT GAA AAT GCA GAC AAA TTG GGC ATT ATC TTG AGA AAT GAA CTC   1080
CGA GTG GCC ATC GCA GCC CTT GAG GTT TTA GAA GAA GAA AAC CTT GCT GAA AAT GCA GAC AAA TTG GGC ATT ATC TTG AGA AAT GAA CTC   1080

Met-Lys-Leu-Pro-Ser-Asp-Val-Val-Thr-Ala-Val-Arg-Gly-Lys-Gly-Leu-Leu-Asn-Ala-Ile-Val-Ile-Lys-Glu-Thr-Lys-Asp-Trp-Asp-Ala   390
ATG AAG CTA CCT TCT GAT GTT GTA ACT GCC GTA AGA GGA AAA GGA TTA TTA AAC GCT ATT GTC ATT AAA GAA ACC AAA GAT TGG GAT GCT   1170
ATG AAG CTA CCT TCT GAT GTT GTA ACT GCC GTA AGA GGA AAA GGA TTA TTA AAC GCT ATT GTC ATT AAA GAA ACC AAA GAT TGG GAT GCT   1170

Trp-Lys-Val-Cys-Leu-Arg-Leu-Arg-Asp-Asn-Gly-Leu-Leu-Ala-Lys-Pro-Thr-His-Gly-Asp-Ile-Ile-Arg-Phe-Ala-Pro-Pro-Leu-Val-Ile   420
TGG AAG GTG TGT CTA CGA CTT CGA GAT AAT GGA CTT CTG GCC AAG CCA ACC CAT GGC GAC ATT ATC AGG TTT GCG CCT CCG CTG GTG ATC   1260
TGG AAG GTG TGT CTA CGA CTT CGA GAT AAT GGA CTT CTG GCC AAG CCA ACC CAT GGC GAC ATT ATC AGG TTT GCG CCT CCG CTG GTG ATC   1260

Lys-Glu-Asp-Glu-Leu-Arg-Glu-Ser-Ile-Glu-Ile-Ile-Asn-Lys-Thr-Ile-Leu-Ser-Phe ***   ***   439
AAG GAG GAT GAG CTT CGA GAG TCC ATT GAA ATT ATT AAC AAG ACC ATC TTG TCT TTC TGAGGGTAGCCAGCTGTTTTCAGTGGTCCCTGGGAGCCAGCTG   1360
AAG GAG GAT GAG CTT CGA GAG TCC ATT GAA ATT ATT AAC AAG ACC ATC TTG TCT TTC TGAGGGTAGCCAGCTGTTTTCAGTGGTCCCTGGGAGCCAGCTG   1360

GAGACAGGTGGTCCTGTAAAAGCTTTATTCCTAATGTGGGCACATTCCACTCCCATGAGTCTTCAAAAACTTTTTTTTTGAATATATTTTTTTCAGTTGATACATAATAGAACAACGTT   1479
GAGACAGGTGGTCCTGTAAAAGCTTTATTCCTAATGTGGGCACATTCCACTCCCATGAGTCTTCAAAAACTTTTTTTTTGAATATATTTTTTTCAGTTGATACATAATAGAACAACGTT   1479

TATGAACCTGCCGTTTGCTTTGTAACGTAACTAAATAATGTAATGGCATCTATATTCAGTTGAAGTGTTTTGATGAATTC   1553
TATGAACCTGCCGTTTGCTTTGTAACGTAACTAAATAATGTAATGGCATCTATATTCAGTTGAAGTGTTTTGATGTGCATGTGTACTTCCTAAGGTGAAATGCATCTATATACAGACAG   1598

CCTCTAAATCAAGTCCTTCAGTATAATTGATATATGTTTTTATAATTTCCTCACTGGTATAAGTGTTTCATATTTGAAAAAGTTATCTCTGGGTATTGCATAAAAGGCTTCATCTTATA   1717

AAGTGAAATCATTGTTATTGAATTTTAGGAAGGATTAATGGTTAAGTGTATATAAAATACTAATATTAAGTAAACTTCATATTGGCCAACACCAGGGTTGTATTCTATGGATGTCATTA   1836

TTTTGAATTAAGAATTAGCGTTTAACATTCCTAAATTGTTTTGAGTGCTTGATTATAATTTGTAAAAAATGTTTATTTTCAATACTTCTTTAAATTTAAAATAAAGCTTATAGTTTCAA   1955

AAAAAAAAAGGAATTC   1971
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Fig.2. Nucleotide and amino acid sequence of human kidney OAT cDNA. The sequence of the 1587-nucleotide-long cDNA insert in the 1.5 kbp hkOAT-cDNA and 1.1 kbp hkOAT-cDNA is presented, as is the amino acid sequence, which is deduced from the open reading frame. The amino acids are numbered starting with the initiating methionine as amino acid number 1. The putative cleavage site between the leader peptide and the purified mature hkOAT is shown by vertical dashes. Terminator codons are indicated by ***.

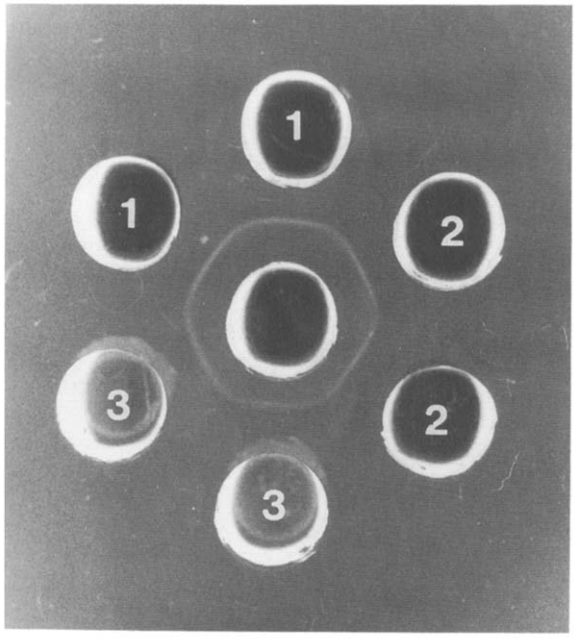

Fig.3. Ouchterlony double diffusion pattern of kidney, liver and brain OAT. Center well contains human kidney OAT antibody (purified IgG). Outer wells contain liver (1), kidney (2) and brain (3) purified OAT antigens. The antibody was raised with purified human kidney OAT as previously reported [16].

Titani, K., personal communication). On the other hand, Inana et al. [7] have postulated that the leader peptide of human OAT was cleaved after Lys-32, based on the apparent molecular mass of the mature OAT protein. Mueckler and Pitot [8] and Simmaco et al. [12] have postulated that the rat OAT precursor was processed after Glu-34 or Ala-25. These differences in the site of cleavage of the OAT precursor may depend on the various different preparations or on the secondary processing.

human	OAT	:	Leu	Gly	Lys	Ala	Leu	Ser	Gly	Gly
rat	OAT	:	Leu	Gly	Lys	Ala	Leu	Ser	Gly	Gly
human	mAAT	:	Cys	Gln	Ser	Tyr	Ala	Lys	Asn	Met
rat	mAAT	:	Cys	Gln	Ser	Tyr	Ala	Lys	Asn	Met
rat	cAAT	:	Ala	Gln	Ser	Phe	Ser	Lys	Asn	Phe
E.coli	AAT	:	Ala	Ser	Ser	Tyr	Ser	Lys	Asn	Phe
rat	TAT	:	Thr	Leu	Ser	Phe	Leu	Lys	Ser	Asn
rat	mSAT	:	Lys	Ser	Lys	Val	Tyr	Ser	Arg	Lys
human	ODC	:	Asp	Glu	Lys	Tyr	Tyr	Ser	Ser	Ser

Fig.4. Comparison of the amino acid sequence of a putative pyridoxal phosphate binding site with five pyridoxal phosphate-requiring enzymes from different species. The mAAT, cAAT and mSAT exhibit mitochondrial AAT, cytosolic AAT and mitochondrial SAT, respectively.

3.3. *Pyridoxal phosphate and L-ornithine binding site*

Pyridoxal phosphate is a coenzyme which is necessary for all aminotransferases, including aspartate aminotransferase (AAT), tyrosine aminotransferase (TAT), serine aminotransferase (SAT), and OAT. Tanase et al. [13] have proposed that the partial amino acid sequence Ser-X-X-Lys is a structural feature common to most of the known pyridoxal phosphate binding sites. Sequences such as Ser-X-X-Lys are present in these aminotransferases and ornithine decarboxylase (ODC), which is another pyridoxal phosphate-requiring enzyme. However, OAT, SAT, and ODC exhibit the right side-left reverse profile, such as Lys-X-X-Ser (fig.4). Hickok et al. [14] have suggested that there is a hydrophilic region in ODC for the binding of L-ornithine. Similarly, OAT and ornithine carbamoyltransferase [15], which bind substrate L-ornithine, also contain hydrophilic regions (amino acid residues 307–343 and 239–282, respectively). Interestingly, these hydrophilic regions in OAT and ODC are flanked with a Lys-X-X-Ser sequence. These regions and the Ser-X-X-Lys or Lys-X-X-Ser sequences may play an important role in the catalytic function of these enzymes, such as the binding of pyridoxal phosphate or L-ornithine.

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