Cloning and Expression of the Mammalian Cytosolic Branched Chain Aminotransferase Isoenzyme*

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The cDNA for the rat cytosolic branched chain aminotransferase (BCAT) 1 has been cloned. The BCATc cDNA encodes a polypeptide of 410 amino acids with a calculated molecular mass of 46.0 kDa. By Northern blot analysis, BCATc message of approximately 2.7 kilobases was readily detected in rat brain, but was absent from liver, a rat hepatoma cell line, kidney, and skeletal muscle. When expressed in COS-1 cells, the enzyme is immunologically indistinguishable from the native enzyme found in rat brain cytosol. Comparison of the rat BCATc sequence with available data bases identified the Escherichia coli (and Salmonella typhimurium) branched chain aminotransferase (BCAT) and revealed a Hae-mophilus influenzae BCAT, a yeast BCAT, which is hypothesized to be a mitochondrial form of the enzyme, and the murine BCAT, (protein ECA39). Calculated molecular masses for the complete proteins are 33.9 kDa, 37.9 kDa, 42.9 kDa, and 43.6 kDa, respectively. The rat BCATc sequence was 84% identical with murine BCAT, 45% identical with yeast, 33% identical with H. influenzae, 27% identical with the E. coli and S. typhimurium BCAT, and 22% identical with the evolutionary related ω-amino acid aminotransferase (ω-AAT) (Tanizawa, K., Asano, S., Masu, Y., Kuramitsu, S., Kagamiyama, H., Tanaka, H., and Soda, K. (1989) J. Biol. Chem. 264, 2450-2454). Amino acid sequence alignment of BCATc with ω-AAT suggests that the folding pattern of the overlapping mammalian BCATc sequence is similar to that of ω-AAT and indicates that orientation of the pyridoxal phosphate cofactor in the active site of the eukaryotic BCAT is the same as in ω-AAT. Thus, BCAT are the only eukaryotic aminotransferases to abstract and replace the proton on the δ face of the pyridoxal phosphate cofactor. Finally, requirements for recognition of substrate ω-amino acid and ω-carboxylate binding are discussed.

In mammals, the branched chain aminotransferase (BCAT) catalyzes the first reaction in the catabolism of the essential branched chain amino acids leucine, isoleucine, and valine. Our laboratory has shown that there are two mammalian BCAT, a cytosolic and mitochondrial isoenzyme, BCATc and BCATm, respectively (Wallin et al., 1990). The rat BCATc is found in brain, where it is the predominant isoenzyme, and in placenta and ovary (Hutson, 1988; Hall et al., 1993; Ichihara, 1985). The second isoenzyme, BCATm, is found in almost all tissues of the body (Hutson et al., 1992). It has been shown that BCATm is a bifunctional protein catalyzing transport of branched chain α-keto acids as well as transamination while BCATc exhibits only transaminase activity (Hutson and Hall, 1993).

Unlike mammals, bacteria appear to have a single BCAT. The two mammalian BCAT isoenzymes are similar to the Escherichia coli and Salmonella typhimurium BCAT with respect to substrate preference and kinetic properties (Inoue et al., 1988; Lee-Peng et al., 1979; Hall et al., 1993); however, the mammalian BCAT appear to be distinct from their ancestral progenitor in both size and quaternary structure. The E. coli and S. typhimurium enzymes are hexamers composed of identical 33.9-kDa subunits (Lipscomb et al., 1974). The mammalian BCAT that have been purified are larger than 40 kDa, and available data suggest that the functional unit of BCATc is a homodimer while the functional unit of BCATm may be a monomer (Wallin et al., 1990; Hall et al., 1993; Ichihara, 1985). No BCAT has been purified yet from lower eukaryotes such as yeast.

Based on primary sequence comparisons with other aminotransferases, it was hypothesized that the prokaryotic BCAT and ω-AAT are members of a unique evolutionary subclass of aminotransferases (Mehta et al., 1993; Tanizawa et al., 1989). Recently, Grishin et al. (1995) have placed these aminotransferases in a separate folding class (fold type IV) and identified 4-amino-4-deoxychorismate lyase and murine protein ECA39 as additional members of this group. Because of its role in providing ω-amino acids for bacterial cell wall synthesis, ω-AAT has been a target enzyme for development of novel antimicrobial agents (Sugio et al., 1995). The crystal structure of ω-AAT, which has just been solved, has revealed that the folding pattern of this protein is completely different from those of other known PLP enzymes (Sugio et al., 1995). As discussed by Sugio et al. (1995), alignment of the bacterial BCAT and ω-AAT amino acid sequences suggests that the folding pattern of the bacterial BCAT resembles that of ω-AAT (Sugio et al., 1995). Understanding the relationship between the mammalian and the bacterial BCAT has been limited to date by the lack of cDNAs for the mammalian enzymes which has precluded structural analysis.

In this study, the cDNA for the rat cytosolic isoenzyme, methylammonio-l-propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; PLP, pyridoxal phosphate.
BCATc has been isolated. The deduced amino acid sequence revealed a 410-amino acid protein with a calculated molecular mass of 46,045 daltons. Sequence comparisons with available data bases identified two additional full-length euukaryotic sequences including the murine BCATc (protein ECA39) and a yeast BCAT as well as a bacterial BCAT from Haemophilus influenzae. The deduced amino acid sequence for rat BCATc shares considerable amino acid sequence identity with the murine and yeast sequences and small, but significant, identity with the bacterial BCAT and p-AAT. Amino acid sequence alignment of BCATc and p-AAT firmly places the euukaryotic enzymes in fold type IV (Grishin et al., 1995) and shows that the evolutionary conservation of key residues involved in cofactor binding as well as provides clues to amino acid residues that may be involved in substrate recognition.

**MATERIALS AND METHODS**

Amino Acid Sequence of Peptides from BCAT. — Approximately 1 nmol of BCATc was purified from rat brain as described previously (Hall et al., 1993). Purified BCATc was precipitated, washed with ethanol, and solubilized in 8 M urea by heating at 37°C for 15 min. The urea was diluted to 50% with 50 mM ammonium acetate, the sample was digested with 1 mol % endopeptidase Lys-C. The resulting peptides were separated by reverse-phase HPLC on an Applied Biosystems Model 140B HPLC system using an Aquapore RP-300 C8 column (2.1 x 100 mm) (Millipore). Peaks from this separation were isolated and sequenced on an Applied Biosystems Model 477A Sequencer by the Protein Analysis Core Laboratory of the Comprehensive Cancer Center of Wake Forest University. Trypsin digestion, peptide separation, and amino acid sequence analysis of purified BCATc (0.5 nmol) were performed at the Harvard Microchemistry Facility (Cambridge, MA).

Synthesis of a Rat BCATc Probe. — The polymerase chain reaction was used to obtain a rat BCATc cDNA probe. First strand cDNA was synthesized from rat brain poly(A)+ RNA using m(7)GTP-3'-dithiothreitol, and purified BCATc; therefore, two separate protein samples were used to obtain the XhoI enzyme digestion map.

**RESULTS**

Immunoblotting, Immunoprecipitation, and Tissue Fractionation — SDS-PAGE was performed according to Laemmli (1970) using 10% (w/v) acrylamide gels. Prior to electrophoresis, samples were boiled for 2 min in the presence of 1% (w/v) SDS and 5% (v/v) ß-mercaptoethanol. For immunoblotting, proteins were transferred to Immobilon P membranes (Millipore) as described previously (Wallin et al., 1990). The filter was blocked with 5% (w/v) bovine serum albumin (Fraction V, Sigma) and incubated with rabbit anti-rat BCATc serum (1:500 dilution) (Hall et al., 1993). Immunoreactive protein bands were visualized using horseradish peroxidase-labeled goat anti-rabbit antibody according to the manufacturer’s instructions (Bio-Rad). For immunoprecipitation, rat brain cytosol (150 μl) or cell extracts (20 μl) were adjusted to 0.5 mM NaCl and mixed with 20 μl of preimmune or isoenzyme-specific anti-BCATc serum in a total volume of 280 μl. Immune complexes were isolated using Staphylococcus aureus Protein A (Pansorbin) as described (Hall et al., 1993).

Rat brain cytosols from whole rat brain and isolated cerebellum, cortex, and hippocampus were prepared as described (Hall et al., 1993). Protein was determined in cytosol fractions and COS-1 cell extracts using the DC Protein Assay (Bio-Rad).

**RESULTS**

N-terminal amino acid sequence could not be obtained from purified BCATc therefore, two separate protein samples were subjected to Lys-C endopeptidase and trypsin digestion, respectively. The amino acid sequence of nine internal peptides is shown in Table I. When the amino acid sequence of these peptides was analyzed, a significant number of the peptides (Cyto 38, 54, 24, 44, 45, and 74; see Table I) were found to align with a previously identified mouse cDNA, ECA39, which was an overexpressed mRNA found in the murine PCC4 Aza1 teratocarcinoma cell line (Niwa et al., 1990). The rat BCATc tryp-
ECA39 sequence revealed that upon shifting the reading frame by the addition of a single nucleotide residue (guanosine) at position 1018, the predicted coding sequence would now align with the three BCATc peptide sequences. The amino acid identity was now greater than 90%, and all of the BCATc peptides, except Cyto 48, could be aligned with the mouse sequence. If a second nucleotide was inserted in the codon at position 1262 to 1263, the deduced ECA39 sequence would now align with Cyto 48 and place Cyto 48 at the C terminus of BCATc. This sequence alignment and expression of ECA39 in brain and kidney (Niwa et al., 1990) strongly suggested that ECA39 is the mouse homolog of BCATc and explains why Grishin et al. (1995) placed it in the same folding class as the E. coli BCAT.

Using the nucleotide sequence of ECA39 and peptide sequence derived from the purified rat BCATc, nondegenerate oligonucleotide primers were designed and used in the polymerase chain reaction with rat brain cDNA. A single product of the expected size (630 base pairs) was obtained. Partial sequence analysis of the product revealed at least 80% nucleotide identity with the mouse candidate BCATc. This product was used to screen a rat brain λgt10 cDNA library (approximately 1 × 10⁸ plaques). Eight positive clones were identified. Six of the original eight plaques were positive by polymerase chain reaction, and two of these clones were plaque-purified and subcloned into pBluescript.

The cDNA and deduced amino acid sequence for BCATc are shown in Fig. 1. The predicted initiator methionine lies in an appropriate consensus for initiation of translation (Kozak, 1987), and is preceded by a 5′-untranslated region of 62 nucleotides. The translation termination codon is followed by a 3′-untranslated region of 72 nucleotides that did not extend to the poly(A) tail. The rat cDNA encodes a protein of 410 amino acids with a calculated molecular mass of 46,045 daltons which is in excellent agreement with the molecular mass of 47 kDa determined by SDS-PAGE (Hall et al., 1993). The translated BCATc sequence contains an alanine residue immediately following the N-terminal methionine. The presence of a modified N-terminal alanine would be consistent with reports characterizing the processing of amino termini of cytosolic eukaryotic proteins (Flinta et al., 1986; Huang et al., 1987) which have shown that when alanine is adjacent to the N-terminal methionine, removal of the initiator methionine residue and modification of the exposed alanine occurs with high frequency. The location of the peptide sequences obtained from proteolytic digestion of purified BCATc in the translated sequence are indicated in Table I. A comparison of the amino acid sequences determined chemically with those determined from the cDNA clone reveals 100% agreement with the tryptic peptide sequences (Cyto 74 and 48) and Cyto peptides 24, 41, and 54. The mismatches in Cyto 44 (His-283) and Cyto 45 (His-312) were correctly determined in Cyto 74 as glycine. Chemically, glutamine was observed at position 344 in Cyto 27, and only one serine instead of two was observed at position 70 in Cyto 38.

BCATc is known to have a unique tissue distribution in the rat. The enzyme is found in brain and is notably absent in other tissues with the exception of low activity in ovary and placenta (Hutson, 1988; Ichihara, 1985; Hall et al., 1993). To compare BCATc expression with the known tissue distribution of BCATc activity and protein, Northern blot analysis was performed with total RNA from whole rat brain (see Fig. 2, lane B). Since the distribution of BCATc throughout the brain is not known, both BCATc expression and cytosol activity were examined in the cerebellum, cortex, and hippocampus (see Fig. 2, lanes marked C, H, and CE). A single mRNA transcript of approximately 2.7 kb was found in all brain regions. The additional length of the mRNA as compared to the length of the cDNA most likely represents an additional 3′-untranslated region. The putative murine BCATc is also encoded by a larger mRNA with a 1107-nucleotide 3′-untranslated region. BCATc activity was found in all brain regions. The ubiquitous expression of BCATc in different brain regions is consistent with reports showing branched chain transamination in astrocyte and neuronal cultures (Hertz et al., 1987; Yudkoff et al., 1994). What has not yet been determined is the exact distribution of BCATc isoforms in different cell types in the brain.

Fig. 3 shows Northern blot analysis performed with total RNA from a rat liver hepatoma (H4-II-E-C3) cell line, rat kidney, liver, and skeletal muscle. Brain RNA is shown for reference. Consistent with previous reports on the tissue distribution of rat BCATc (Hutson, 1988; Ichihara, 1985; Hall et al., 1993), no hybridization was observed with RNA from these tissues, even after lower stringency hybridization conditions. Stripping and reprobing the blots with a probe for glycoaldehyde-3-phosphate dehydrogenase (Ercolani et al., 1988) confirmed the load and integrity of the RNA (Figs. 2 and 3, lower panels).

To demonstrate functional expression of BCATc, BCATc cDNA was ligated into an expression vector (pBcatc) and transfected into COS-1 cells. The expressed BCATc protein was visualized by immunoblotting using an antibody raised against purified rat BCATc (Hall et al., 1993) (see Fig. 4A). Cell extracts were also assayed for BCATc activity and immunoprecipitated with BCATc antisera (see Fig. 4B). Lane 5 (pBcatc) contains the pBcatc-transfected COS-1 cell extract, and lane 1 extract from mock-transfected COS-1 cells (pGAL). Lanes 2–4 contain rat brain cytosol preparations (Brain Cytosol) as a reference. A band of about 47 kDa was readily detected in the rat brain cytosols and in the pBcatc-transfected COS-1 cell extract. The appearance of the lower band in lane 5 varied according to the composition of the inhibitor mixture which indicated that the lower band resulted from proteolytic degradation. The same observation was made when the enzyme was purified from rat brain.
brain cytosol which suggests that the protein is very sensitive to proteolysis (Hall et al., 1993). COS-1 cell BCAT specific activity was 26-fold higher (range 20- to 100-fold in four separate transfection experiments) than in brain cytosol and essentially all BCAT activity was neutralized by the BCATc antiserum (see Fig. 4 B, pBCATc). Neither BCATc antigen (see Fig. 4A, lane 1) nor significant BCAT activity (see Fig. 4B, pβGal) was observed in the extract from the pβGal-transfected cells.

The relative amino acid substrate specificity of BCATc expressed in COS-1 cells with KIC as amino acceptor was also examined. The amino acid preference in descending order was isoleucine, leucine, valine, norvaline, glutamate, and norleucine, which is essentially the same as has been reported previously for purified BCATc (Hall et al., 1993). Phenylalanine and alanine were not transaminated (≤1% of control value with leucine as amino acceptor).

In addition to the murine ECA39 cDNA, a search of the available protein and nucleic acid databases revealed that the rat BCATc protein sequence has significant similarity to a yeast sequence, and partial sequences from the nematode Caenorhabditis elegans, the plant Arabidopsis thaliana, several human fragments as well as the bacterial BCAT from E. coli and S. typhimurium and a gene product from H. influenzae Rd (Fleischmann et al., 1995). A multiple sequence alignment of the rat, mouse, yeast, and bacterial BCAT sequences is shown in Fig. 5. Since only 7 of 308 residues from the genetically

![Fig. 1. Nucleotide and predicted amino acid sequence of the rat BCATc.](http://www.jbc.org/)

![Fig. 2. Northern blot hybridization of RNA from rat brain.](http://www.jbc.org/)
DISCUSSION

This manuscript presents the first cloning of a mammalian BCAT. The tissue distribution of the BCAT\(_c\) mRNA is consistent with the known distribution of the cytosolic BCAT isoenzyme determined by measurements of activity and immunological techniques. The protein, when expressed in COS-1 cells, is immunologically indistinguishable from the enzyme in rat brain cytosol, and the amino acid substrate preferences were the same as the enzyme purified from rat brain cytosol (Hall et al., 1993).

Two additional complete eukaryotic BCAT sequences have been identified. The ECA39 cDNA (Niwa et al., 1990), once two nucleotide changes are made, appears to code for the murine BCAT\(_c\) with a calculated molecular mass of 42.9 kDa. The other sequence is a yeast BCAT with a calculated molecular mass of 43.6 kDa for the complete protein. Very little is known about the yeast BCAT including whether or not these lower eukaryotes contain both isoenzymes. However, the calculated isoelectric point for the yeast enzyme of 5.59 is considerably higher than the calculated values of 5.6 and 5.25 for the rat and mouse cytosolic enzymes, respectively. Hartmann and Christen (1991) observed that, with few exceptions, the pI of the mitochondrial isoenzyme has a much higher isoelectric point than the cytosolic form of the same enzyme. If the yeast enzyme is a mitochondrial protein, then it is likely to have an N-terminal targeting sequence. The alignment of the mammalian BCAT with the yeast sequence becomes unambiguous at BCAT\(_c\)-51 (Yeast BCAT-25) and Leu-71 in BCAT\(_c\) (Yeast BCAT-45) is the start of a highly conserved sequence (LVFG in Fig. 5). Mitochondrial targeting signals are generally from 10–70 amino acids, can form an amphipathic helix composed of positively charged and hydrophobic residues and generally lack acidic amino acids (Hartl et al., 1989). An arginine is usually found at the 2 position from the mitochondrial processing peptidase cleavage site (Hartl et al., 1989). Secondary sequence analysis shows potential helical structure in this region, and an \(\alpha\) helix would be amphipathic. Only one acidic residue, an aspartic acid, is found in the first 34 amino acids. Based on the alignment shown in Fig. 5 and assuming an arginine at the 2 position, cleavage could occur in the yeast BCAT between residues 16 and 17 resulting in a mature protein of 377 amino acids with a calculated molecular mass of 41 kDa. Two other potential cleavage sites preceding BCAT\(_c\)-Leu-71 appear less likely since they occur after BCAT\(_c\)-51.

Cloning of the first mammalian BCAT, rat BCAT\(_c\), and identification of two additional full-length eukaryotic sequences, is the first step in developing a molecular model for the eukaryotic BCAT. Sequence similarity between the E. coli BCAT and \(\delta\)-AAT led to the hypothesis that these two enzymes evolved from a common ancestral gene (Tanizawa et al., 1989). The enzymes are 25% shorter than the average aminotransferase length of approximately 400 amino acids, and alignment of these enzymes with other aminotransferases indicates that the small domain as defined in aspartate aminotransferase is

\(^2\) R. Bledsoe, P. A. Dawson, and S. M. Hutson, unpublished results.
either absent or of different structure. Another unique property of these enzymes concerns the stereochemistry of the hydrogen transfer which occurs during catalysis on the re face of the cofactor while in other classes of aminotransferases proton transfer occurs from the si face (Yoshimura et al., 1993). The crystal structure of the pyridoxamine-form of thermostable D-AAT has been solved, and it is clear that the structure is distinct from the other PLP-containing enzyme structures that have been reported (Sugio et al., 1995). Fig. 6 presents a GAP alignment of rat BCATc and D-AAT. Structural analysis of D-AAT shows that, unlike other aminotransferases, the N-terminal domain (D-AAT residues 1–120)
and the C-terminal domain (D-AAT residues 121–282) are comprised of very different secondary and tertiary structures, and the pyridoxal phosphate binding site is located mainly at the interface of the two domains of the same monomeric rather than being shared between the two monomers as in aspartate aminotransferases (Sugio et al., 1995). Only two residues, Arg-98* and His-100* from the other subunit, extend to the active site and PLP-binding region. Secondary structure predictions of the overlapping regions of rat BCATc and D-AAT show a similar pattern, suggesting that the two proteins have similar folding patterns. Therefore, the mammalian BCAT can be classified as belonging in the folding group IV described by Grishin et al. (1995).

Since the components necessary for transamination are clearly present in the shortest bacterial BCAT, the function of the N-terminal region of the eukaryotic enzymes is not known. Except for the conserved sequence around BCATc-71, the N-terminal portions of the eukaryotic BCAT are the most variable regions of these sequences (see Fig. 5). Secondary structure predictions for the N terminus of BCATc are predominantly a-helix, suggesting an ordered structure. Since the quaternary structure of rat BCAT, and the E. coli BCAT appears to be different, dimer versus hexamer, the possibility exists that the N-terminal extension may influence quaternary structure. (The H. influenzae RdBCAT appears to be of intermediate size, but the protein’s quaternary structure is not known.) Another possibility is that the small highly conserved portion of this region (BCATc-71 to -82) serves a function analogous to the extended amino segment of the aspartate aminotransferases and stabilizes the dimer (Fukumoto et al., 1991). The N terminus may also play a role in domain movement (Kirsch et al., 1984).

In the transamination reaction the catalytic lysine has an important role in determining the stereochemistry of the proton transfer (Kirsch et al., 1984; Sugio et al., 1995). In L-aspartate aminotransferase, the lysine faces the side of the substrate where the a-proton is located (see Fig. 7A). The lysine is the general base involved in abstraction of the proton from the a-carbon and reprotonation of the aldehydic carbon of the coenzyme to yield a ketimine intermediate. The lysine then returns the proton onto the pro-S side of the substrate so that the L-configuration is maintained when the reaction proceeds.
in the reverse direction. The lysine approaches the coenzyme from the si face which is the side facing the protein. In d-AAT and E. coli BCAT, the proton is added to or abstracted from the C4’ atom of the coenzyme-imine or external aldimine intermediate on the reverse direction (Yoshimura et al., 1993). The crystal structure of d-AAT shows that the cofactor is bound so that the side chain of the catalytic lysine (d-AAT Lys-145) and the α-proton point toward the protein as in L-aspartate aminotransferase (see Fig. 7B). Key active site residues involved in cofactor binding in d-AAT are conserved in the bacterial BCAT suggesting a similar cofactor orientation (Fig. 7C). The GAP alignment shown in Fig. 6 indicates that these structural features are conserved in rat BCAT, and also in the other BCAT sequences (see Fig. 5). The key residues in d-AAT that are also found in BCAT, include the active site lysine (d-AAT Lys-145), d-AAT Glu-177, which interacts with the pyridoxal nitrogen, and the residues which interact with the phosphate group, d-AAT Ile-204, Thr-205, Thr-241, and Arg-50 (Sugio et al., 1995) (see boxed residues in Fig. 6). Since it is the main chain amide of d-AAT Ile-204 that forms the hydrogen bond with a phosphate oxygen, substitution of a Val (BCATc-313) in this position should not perturb the structure significantly. The helix dipole (N terminus of helix d-AAT 203-216) which interacts with the negative charge on the phosphate may also be conserved in the BCAT (see Fig. 6). Secondary structure predictions for the equivalent residues in BCATc residues 312-325, suggest that BCATc and d-AAT would have similar secondary structure in this region. d-AAT Leu-201, which is about 3.9 Å from the C4’ of PLP, is also conserved in BCATc (Leu-310 in Fig. 6) and the other BCAT sequences (see Fig. 5). The d-AAT L201A and L201W mutants show anomalous kinetics, and the enzymes appear to form an inactive pyridoxamine form during catalysis (Kishimoto et al., 1995). In addition, two other residues conserved in fold class IV enzymes, d-AAT Gln-181 and Phe-183, are also found in BCATc. Therefore, it seems reasonable to conclude that orientation of the PLP cofactor in the eukaryotic BCAT is the same as in d-AAT (see Fig. 7, B and C).

Two elements of the PLP binding site in d-AAT may be altered in the BCAT enzymes. Tyr-31 in d-AAT which appears to be in a position to bind to the phenolic oxygen (O3’) and imine nitrogen atom of PLP is replaced by phenylalanine in all BCAT (see Figs. 5 and 6). The equivalent tyrosine in L-aspartate aminotransferase plays an important role in stabilization of intermediates during transamination (Kirsch et al., 1984; Arnone et al., 1985). Whether or not the tyrosine in the adjacent conserved motif beginning at BCATc Leu-112 (LHYxx(L,V,C)FEG) would be in a position to hydrogen-bond the phenolic oxygen remains to be established. A second difference in d-AAT and aspartate aminotransferase is in the residues that form a parallel stacking interaction with the pyridoxal ring. The tryptophan residue that serves this function in aspartate aminotransferase is absent in d-AAT. Instead, the side chain hydroxyls of a loop of three serine residues (D-AAT Ser-179 to Ser-181) hydrogen-bonded to the carboxylate group.

Fig. 7. Schematic diagram of the orientation of the substrate amino acid, active site lysine, and pyridoxal phosphate (PLP) cofactor in L-aspartate aminotransferase (ASP-AT), d-amino acid aminotransferase (d-AAT), and L-branched chain aminotransferases (BCAT). The cofactor is positioned as seen in the structure of porcine ASP-AT, d-AAT, and the predicted position in the rat BCAT. The cofactor side facing solvent is toward the viewer and the si face facing the protein (ASP-AT), and, in d-AAT and BCAT, the re face is facing the protein. The incoming α-amino acid reacts with the internal aldimine between the active site lysine and PLP, and the α-proton faces the protein.
able, because of the opposite orientation of an \( \alpha \)-amino acid and \( \alpha \)-amino acid in the active site (see Fig. 7, B and C); hence, this region of the BCAT is probably involved in side chain recognition. Modeling of E. coli BCAT on the \( \alpha \)-AAT structure that is described, but not shown in Sugio et al. (1995), led to the proposal that either the Arg present in the E. coli sequence equivalent to residue \( \alpha \)-AAT-35 or \( \alpha \)-AAT-88 could be located on the proper side of the active site for \( \alpha \)-carboxylate binding in the bacterial enzyme. Examination of the alignment of BCAT\(_{E} \) with \( \alpha \)-AAT in Fig. 6 and the consensus sequence in Fig. 5 does not reveal a conserved arginine residue at either position in the BCAT sequences although four arginines are found in the consensus sequence shown in Fig. 5. However, there is a conserved arginine at BCAT\(_{C}-187 \) (equivalent to \( \alpha \)-AAT-83) and an arginine that is found in all BCAT sequences except the E. coli enzyme at BCAT\(_{C}-126 \) (equivalent to \( \alpha \)-AAT-38) (see Fig. 6). Mutagenesis will be required to test these candidate arginines.

In summary, cloning of the eukaryotic BCAT has confirmed placement of the BCAT in the same folding group as \( \alpha \)-AAT and the bacterial BCAT (Grishin et al., 1995). The mammalian proteins, however, have acquired additional amino acid sequence during evolution, a structural change that has not been observed in the other aminotransferase subclasses (Mehta et al., 1993). Preliminary modeling of the mammalian BCAT on the \( \alpha \)-AAT structure (data not shown) has provided some insights into regions that may be important in the structure of the BCAT active site which can be tested by site-directed mutagenesis.

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