Branched-Chain Amino Acids: Enzyme and Substrate Regulation

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ABSTRACT  The three branched-chain amino acids (BCAAs) are the most hydrophobic of the amino acids and play crucial roles in determining the structures of globular proteins as well as the interaction of the transmembrane domains of membranous proteins with phospholipid bilayers. However, the three BCAAs do not behave identically. In terms of protein secondary structure, valine and isoleucine exhibit a definite preference for the $\beta$-structure, whereas leucine has a higher preference for the $\alpha$-helix. Although mutation of one BCAA to another is commonly regarded as conservative, there are well-documented examples of such substitutions that have a significant effect on protein function. The occurrence of BCAA in nature is, therefore, attributable to their primary role in protein structure, not to their secondary metabolic roles. These functions are important for almost all proteins; therefore, BCAA commonly account for ~20–25% of most dietary proteins. Dietary BCAA largely escape first-pass splanchnic metabolism. The first steps in their catabolism are common to all three, involving the BCAA aminotransferase (BCAT) and branched-chain $\alpha$-keto acid dehydrogenase (BCKD). Their further metabolism employs distinct pathways to different end-products (glucose and/or ketone bodies). However, the fact that the flux-generating step for the catabolism of the three BCAAs occurs at one of the common steps indicates that the production of these downstream products are not individually regulated and, hence, may not play important individual roles. The catabolism of the BCAAs is highly regulated by both allosteric and covalent mechanisms. BCKD is inhibited by phosphorylation and activated by dephosphorylation. Allosteric inhibition of the kinase by the branched-chain keto acids (BCKA) (particularly by $\alpha$-ketoisocaproate) serves both as a mechanism for promoting the catabolism of excess quantities of these amino acids as well as for conserving low concentrations of these dietary essential amino acids. Cytosolic and mitochondrial isoenzymes of BCAT have been identified. They are thought to play an important role in brain neurotransmitter metabolism. J. Nutr. 136: 207S–211S, 2006.

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The metabolism of BCAAs presents a number of novel features: the catabolism of these three amino acids is controlled by a common flux-generating step, their catabolic disposal occurs largely in skeletal muscle, their circulating concentrations can influence the brain uptake of precursor amino acids for neurotransmitter synthesis, and they can regulate protein synthesis in a variety of tissues. Despite these unusual, or even unique, features it is quite certain that the most important function of these amino acids lies in their roles in proteins. In this article we discuss the role of the BCAAs in protein structure, their metabolic disposal, the key enzymes of their metabolism, and the regulation of these enzymes.

BCAAs AND PROTEIN STRUCTURE

Why BCAAs?  Leucine, isoleucine, and valine are among the most hydrophobic of amino acids. In fact, by some measures of hydrophobicity they are the most hydrophobic (1). This is a crucial determinant of their role in globular proteins, membranous proteins, and coiled-coil structures. Generally, the interior of water-soluble globular proteins consists, largely, of hydrophobic amino acids, principally leucine, isoleucine, valine, phenylalanine, and methionine. This is important, not only for the stability of the folded protein, but also for the folding pathway that leads to the mature structure (2). It is also important for the function of some globular proteins; for example, the hydrophobic residues create a nonaqueous environment that is important for oxygen binding in myoglobin and hemoglobin, and for substrate binding and catalysis in a variety of enzymes (3). A few globular proteins are not water soluble, but lipid soluble. For example, lung surfactant protein B must...
interact with phospholipids in surfactant, so it is among the most hydrophobic of proteins. BCAAs make up 37% of the amino acid composition of surfactant protein B (17.7% leucine, 11.4% valine, 7.6% isoleucine) (4). The dominant structural feature of this protein is its amphipathic helices, with the branched-chain residues interacting with lipid acyl chains, and positively charged groups interacting with the lipid head groups (4). Membranous proteins require hydrophobic amino acids in their transmembrane domains for interaction with the hydrocarbon chains of fatty acids. For example, glycophorin, a protein in the red cell membrane, contains a total of 11 BCAAs in the 19 residues of the α-helix that crosses the membrane (5).

Another important role of leucine and its hydrophobic partners occurs in coiled-coiled α-helices in such proteins as myosin, fibrinogen, keratin, and a number of transcription factors. Each polypeptide of the rod-like coiled coil has an amino acid sequence containing a number of heptad repeats, with leucine often in the fourth position, and another hydrophobic residue in the first position. When the helices twist, there are 3.5 residues per turn of the helix and the helices are packed together by hydrophobic interactions between the leucyl residues and the residues in the 1 position (6). In transcription factors, coiled coils, referred to as leucine zippers, permit formation of homodimers and/or heterodimers, which are the functionally active form of such transcription factors as Fos and Jun (7).

Although a variety of weak interactions (hydrogen bonds, salt-linkages, van der Waals interactions, hydrophobic interactions) are key determinants of protein structure, their relative importance is not invariant. The proteins of thermophilic organisms, in particular, their relative resistance to unfolding at high temperatures, have been a source of fascination for protein chemists. Undoubtedly, many factors play a role in this thermotolerance: among them, it has been suggested that hydrophobic interactions may be particularly important as the strength of these interactions increases with increasing temperature (8). A comparison of the amino acid composition of 110 pairs of homologous proteins from mesophilic and thermophilic organisms showed that the thermophilic proteins have both higher hydrophobicity and charged amino acid composition; the increase in hydrophobicity was largely accounted for by their higher leucine composition (9).

Why three BCAAs? Despite their similarity, the three BCAAs play subtly different roles in proteins. Their side-chains differ in size, shape, and hydrophobicity. Therefore, they have different predilections for different secondary structure motifs. Leucine is more common in α-helices than in β-sheets, whereas the reverse is true for valine and isoleucine (10). These preferences account for the key role of leucine in helical zipper structures. The differences also account for the fact that these amino acids are not always interchangeable in proteins. There are, of course, many occasions when such substitutions are conservative but this is not invariant. For example, a substitution of isoleucine for valine (V122I) in transthyretin results in amyloid deposition in the heart (11). A valine for leucine substitution (L162V) in the pepsinogen proliferator-activated receptor-α results in an altered plasma lipid profile (12) whereas a leucine for valine substitution (V836L) in the extracellular calcium receptor of the TM6/TM7 protein leads to autosomal dominant hypocalciuria (13).

All three of the BCAAs are readily produced under conditions designed to mimic prebiotic organic synthesis (14). They have been retained because of their critical roles in protein structure. Most proteins have a relatively high proportion of these amino acids. Indeed, most dietary proteins consist of ~20% BCAAs. They comprise some 35% of the indispensable amino acid requirements of mammals (15).

**METABOLIC DISPOSAL OF THE BCAAS**

**Unusual aspects of BCAA metabolism.** BCAA metabolism is characterized by a number of unusual features. First, although these amino acids play a number of regulatory roles (e.g., in muscle protein synthesis, insulin secretion, and brain amino acid uptake), they are not metabolized to unique biologically active molecules. Second, their catabolism involves two common steps and, indeed, the flux-generating step, branched-chain keto acid dehydrogenase (BCKD), is a common step (Fig. 1)(16); therefore, BCAAs tend to be catabolized in lockstep. Third, dietary BCAAs largely escape first-pass hepatic catabolism.

**Tissue distribution of BCAA catabolism.** The capacity of different tissues to catabolize BCAAs in humans is shown in Figure 2. This capacity was calculated by multiplying the activities of the branched-chain amino acid aminotransferase (BCAT) and the BCKD in different tissues (17) by the mass of these tissues in a 70 kg human (18). In the case of the BCKD, only the active component was computed. The results are striking. It is clear that one of the distinguishing features of BCAA catabolism is the relatively small fraction of the capacity that resides in the liver. About half of the capacity resides in skeletal muscle, whereas a considerable portion of the activity also resides in adipose tissue. These results are only suggestive because other factors such as rates of blood flow, and transport into cells, are also important. Nevertheless, they are consistent with a body of experimental evidence. For example, Wahren et al. (19) showed that after ingestion of a protein-rich meal the three BCAAs accounted for >50% of the splanchic output of amino acids even though they only comprise 20% of the protein source (beef) that was ingested. Clearly, they largely escaped splanchic catabolism. Elia and Livesey (20) examined human forearm metabolism after ingestion of a steak. They observed a marked increased in BCAA uptake into muscle, but only a minor increase in BCKA output. A similar observation was made after leucine infusion. Clearly, muscle is a major site for BCAA catabolism.

Why should the BCAAs be catabolized extra-hepatically? Liver contains the urea cycle as well as the catabolic enzymes for most of the amino acids. However, it is now apparent that the textbook statement that liver oxidizes most of the dietary amino acids cannot be true. The oxidation of 100 g of dietary protein obliges the consumption of ~3.8 mol of O₂ per d. However, the liver only consumes ~3 mol of O₂ per d (20), so that even if no other dietary substrate were consumed by the liver (i.e., no carbohydrate, fat, or alcohol) it would be impossible to completely oxidize dietary amino acids to CO₂ and H₂O. It is now apparent that this problem is resolved in two ways. First, the liver does not completely oxidize many amino acids but converts them to glucose and acetoacetate, even in the fed state (21). Second, a considerable portion of dietary amino acids are metabolized extra-hepatically, including the appreciable first-pass intestinal metabolism of many amino acids (22) and the muscle catabolism of the BCAAs.

**Consequences and implications of the common steps in BCAA catabolism.** The second unusual feature of BCAA metabolism is that the first two enzymes of their catabolic

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**Fig. 2.** Tissue distribution of BCAA catabolism. The capacity of different tissues to catabolize BCAAs in humans is shown in Figure 2.
disposal, the aminotransferase and the flux-generating dehydrogenase, are common to the three amino acids (Fig. 1). This, no doubt, accounts for the remarkable correlation among the plasma levels of the three amino acids in a variety of situations, such as in Type 1 (23) and Type 2 diabetes (24) (Fig. 3). These data suggest that the plasma concentrations of the individual BCAAs are not tightly defended and therefore not particularly important. Nor does the catabolic fate of the different BCAAs appear to be an important consideration. Leucine is ketogenic, valine is glucogenic, and isoleucine is both glucogenic and ketogenic. However, the fact that the catabolism of all three BCAAs is regulated at a common step suggests that it is not primarily driven by a need for glucose or a need for ketone bodies.

Interorgan aspects of BCAA metabolism. Muscle is not a gluconeogenic tissue. Therefore, if valine and isoleucine are to be converted to glucose they cannot be completely oxidized in this tissue. Our own work with [1-14C]- and [U-14C]-α-ketoisocaproate has shown that this keto acid is almost completely oxidized in the perfused rat heart (25). However, similar experiments showed that α-ketoisovalerate is not; we identified β-hydroxyisobutyrate (an intermediate in the valine catabolic pathway) as a major end product of α-ketoisovalerate catabolism. Lee and Davis (26) obtained similar results in the isolated perfused hind-limb. Plasma levels of β-hydroxyisobutyrate are reported to be 21 μM and 97 μM, respectively, in control and 3-d fasted humans (27). A special feature of the valine catabolic pathway is that CoA is hydrolyzed off one of the intermediates (β-hydroxyisobutyryl-CoA) and subsequently, reintroduced at the level of methylmalonic semialdehyde. Shimomura et al. (28) view this as a means of reducing the concentration of methylacrylyl CoA, which is a highly reactive intermediate. Additionally, β-hydroxyisobutyrate can serve as an inter-organ gluconeogenic substrate. We have shown that β-hydroxyisobutyrate is a good glucogenic substrate in both hepatocytes and renal cortical tubules (29). These studies extend the classic work of Chang and Goldberg (30), with isolated diaphragms, that show isoleucine and valine as major carbon sources for glutamine synthesis.

ENZYME EXPRESSION AND REGULATION

Branched-chain amino acid aminotransferase. Work from Hutson’s laboratory has shown that there are two BCAT isoenzymes, one located in mitochondria and the other in the cytosol (17). These are different gene products. In rats, the mitochondrial isoenzyme is expressed ubiquitously, whereas the cytosolic activity is restricted to brain, ovary, and placenta (31).
In humans, the mitochondrial activity is widely distributed, with particularly high expression in the colon, kidney, and skeletal muscle and a very low expression in the liver (17). Of human tissues examined, cytoplasmic activity was found to be expressed only in the brain; however, ovarian and placental tissues were not examined (17). A marked induction of the mitochondrial BCAT in mammary epithelial cells has been found during late pregnancy and lactation in the rat (32).

The mitochondrial BCAT isoenzyme is primarily responsible for initiating BCAA catabolism. Hutson et al. (33) have made a novel proposal for the function of the cytosolic isoenzyme in brain. A BCAA-based nitrogen shuttle is hypothesized that would play an important role in glutamergic neurons (Fig. 4). Glutamate is the major excitatory neurotransmitter in the central nervous tissue. Upon release into synapses, it initiates neurotransmission and is then efficiently taken up by astroglial cells, which convert it to glutamine, via glutamine synthetase. This glutamine is released to neurons that employ glutaminase to convert it back to glutamate, thus completing the cycle. However, this cycle is not 100% efficient, and some of the glutamate is oxidized in the astroglia and must be replaced, via anaplerotic mechanisms involving pyruvate carboxylase. α-Ketoglutarate, produced in astroglial mitochondria in this process, is transaminated to glutamate by the mitochondrial BCAT. The branched-chain keto acids (BCKAs) produced are thought to be transferred to the neurons where they can be transaminated back to BCAA by the cytoplasmic BCAT. The ultimate source of neuronal amino groups is ammonia, which is fixed into glutamate by glutamate dehydrogenase.

Two pieces of evidence support such a shuttle. First, in the central nervous system, the mitochondrial BCAT is neuronal, whereas the cytosolic isoenzyme is found in the astroglia. Second, gabapentin, an inhibitor of the cytoplasmic BCAT, inhibits the transamination of leucine and the de novo synthesis of glutamate and glutamine in rat retinal preparations (17). Hutson’s group has extended its studies of these enzymes to a variety of organs and to peripheral nerves (34). The important findings are that the mitochondrial BCAT co-localized with BCKD (very high expression of both enzymes were found in various secretory cells), whereas the cytosolic BCAT occurred in cells that do not express BCKD. This localization of the cytosolic isoenzyme certainly points to a physiological role other than in BCAA oxidation.

**Branched-chain keto acid dehydrogenase.** BCKD is the flux-generating step for BCAA catabolism. Classic isotopic work in humans, by Matthews et al. (35), showed that leucine transamination was much faster than the decarboxylation of α-ketoisocaproate. This irreversible oxidative decarboxylation is catalyzed by an enzyme complex, the BCKD complex, which consists of multiple copies of the BCKA decarboxylase (E1), dihydrolipoamide acyltransferase (E2), and dihydrolipoamide dehydrogenase (E3). A genetic defect in the BCKD complex is responsible for maple syrup urine disease. The activity of the BCKD is regulated by both covalent and allosteric mechanisms. Phosphorylation of E1 (on its α subunit) by the BCKD kinase inhibits its activity; this may be reversed by dephosphorylation via a phosphatase. The BCKD kinase has been isolated, characterized, and cloned (36) and is considered to be the key regulator of BCKD activity, although this perception may need to be modified when more is known about the phosphatase. The kinase is regulated in two different ways. It is allosterically...
inhibited by α-ketoisocaproate (and by the other BCKAs, although they are less effective), which provides an elegant means of enhancing BCAA disposal when they are present in excess and of conserving these essential amino acids when they are less available. This effect of α-ketoisocaproate also explains the finding that the provision of excess leucine results in decreased circulatory levels of valine and isoleucine. The BCKD kinase also appears to be regulated by its association with the BCKD complex, although the molecular details of this interaction have not yet been established. Shimomura et al. (16) have reviewed the regulation of BCKD kinase expression by nutritional, hormonal, and pathological factors. The active dephosphorylated BCKD is also susceptible to allosteric inhibition, in particular by NADH and by the CoA esters that arise during BCAA catabolism.

**PERSPECTIVE**

How well do we understand BCAA metabolism?

We have learned a great deal about BCAA metabolism, both in vivo and in vitro, in recent years. Although it is regarded as a mature field of study, we may wonder at the extent of our knowledge. The occurrence of the diseases hypervalinemia (37) and hyperleucineemia-hyperisoleucinemia are, apparently, due to the impaired transamination of valine alone, or of both leucine and isoleucine. Such disorders challenge the conventional view that each of the BCAAs is transaminated by a common aminotransferase. It seems likely that these diseases are due to mutations in the common aminotransferase and that they affect its ability to act on each of the BCAAs. Nevertheless, this has not been established.

Is our knowledge of BCAA metabolism sufficient to construct a mathematical model and, if so, how well will it accommodate experimental observations? Our knowledge of the kinetic properties of the components of BCAA catabolism is probably sufficient to permit the construction of a robust model. After decades of biochemical reductionism we should aim to reconstruct the system. Such reconstructions can be very revealing. For example, modeling of a linear pathway of yeast glycolysis, with all the known in vitro kinetic properties of the constituent enzymes, failed to predict a stable steady-state. However, a closer match of the model to the experiment could be attained by the inclusion of branches, such as toward glycogen, trehalose, etc. (38). In this era of Systems Biology, it behooves us to subject our knowledge of BCAA metabolism to a comparable test.

**LITERATURE CITED**
