Physiological and pathological roles of branched-chain amino acids in the regulation of protein and energy metabolism and neurological functions

Yoshiharu Shimomura*, and Yasuyuki Kitaura

Lab. of Nutritional Biochemistry, Department of Applied Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan

*Corresponding author:

Lab. of Nutritional Biochemistry, Department of Applied Biosciences,
Graduate School of Bioagricultural Sciences, Nagoya University,
Chikusa-ku, Nagoya 464-8601, Japan

E-mail address: shimo@agr.nagoya-u.ac.jp for Y. Shimomura,
ykitaura@agr.nagoya-u.ac.jp for Y. Kitaura

Abstract

Branched-chain amino acids (BCAAs: leucine, isoleucine, and valine) are essential amino acids for humans and play an important role as the building blocks of proteins. Recent studies have disclosed that free BCAAs in the tissue amino acid pool function not only as substrates for protein synthesis, but also as regulators of protein and energy metabolism. Furthermore, BCAAs are actively used as an amino group donor to synthesize glutamate in the brain. These functions of BCAAs are closely related to human health. This review summarizes the recent findings concerning physiological and pathological roles of free BCAAs in the metabolism and neurological functions.

Keywords: Branched-chain amino acids; branched-chain α-keto acid dehydrogenase; protein metabolism; glucose metabolism; energy metabolism; neurological development.

1. Introduction

Branched-chain amino acids (BCAAs: leucine, isoleucine, and valine) are essential amino acids for protein synthesis in humans. The content of amino acids in animal proteins is as high as ~20%, and the BCAA composition (Leu:Ile:Val) in the proteins is found at an approximate ratio of 2:1:1. The high ratio of leucine might reflect its function as the most physiologically potent amino acid among the three BCAAs. In contrast to the high BCAAs content in proteins, the concentrations of free BCAAs in the amino acid pool of human skeletal muscle are as low as ~0.65 mM, and have an approximate ratio of 2:1:3 [1]. The levels of BCAAs in the amino acid pool under post-absorptive conditions are relatively stable [2]. Ingestion of proteins and BCAA supplements rapidly increases the concentrations of free BCAAs in the amino acid pool, with plasma BCAA concentrations reportedly peaking ~30 min after BCAA supplement intake [2]. This rapid rise of BCAA concentrations in the amino acid pool exerts the physiological effects of amino acids on protein and glucose metabolism. In this review, we summarize characteristics of the regulation of BCAA catabolism and the recent findings regarding the physiological functions of free BCAAs.

2. Regulation of BCAA catabolism

The BCAA catabolic system is equipped in mammalian cells to dispose of excess BCAAs, presumably resulting in relatively stable concentrations of BCAAs in blood and tissues in humans. The main BCAA catabolic pathway is localized in the mitochondria of all tissues. The first 2 steps of the catabolic pathway are common to the three BCAAs (Fig. 1) [3].

The first step reaction is the transamination of BCAAs to form

branched-chain α-keto acids (BCKAs), which is a reversible reaction catalyzed by branched-chain aminotransferase (BCAT). Two isozymes of BCAT have been reported; one is a mitochondrial type (BCATm or BCAT2) that is expressed in all of the peripheral tissues except for liver, and the other one is a cytosolic type (BCATc or BCAT1) that is unique to cerebral tissue, placenta, and ovaries [4].

The second step reaction is the oxidative decarboxylation of BCKAs to form CoA esters, which is catalyzed by BCKA dehydrogenase (BCKDH) complex (BCKDC). This reaction is irreversible, and it is therefore thought that BCAA catabolism is regulated by BCKDC. The activity of BCKDC is quite high in the liver of rodents compared to other organs, even though BCATm is generally not expressed in the liver [5]. BCKDC is regulated by covalent modifications: the complex is inactivated by phosphorylation of the E1 component of BCKDC and reactivated by dephosphorylation of the component. BCKDH kinase (BDK) [6,7] and BCKDH phosphatase [8,9] are responsible for these modifications, respectively. These reactions allow the rapid conversion of the activity state of BCKDC in response to alterations in the nutritional and physiological conditions of the animals. BDK is the first mitochondrial protein kinase to be cloned and its amino acid sequence indicates that it is more closely related to prokaryotic histidine kinases than to eukaryotic serine/threonine protein kinases [7].

The transamination of BCAAs by BCATm in the normal mammalian body under resting conditions is largely conducted in skeletal muscle, attributable to the relatively high enzyme activity in the tissue [5] and the large tissue mass (~40% of body weight of humans) in the body. However, BCATm may mostly act at the postprandial state, because of the high *K*m values of BCATm (0.6-3 mM) compared to the BCAA concentrations at the postabsorptive state [10]. In contrast to BCAT, the activity of

BCKDC in skeletal muscle is very low, although the affinity of BCKDC to BCKAs is very high (*K*m: 20-40 μM). The low activity of BCKDC in skeletal muscle is attributed to the high activity of BDK in the tissues [11] and may contribute to conserve BCAAs for protein synthesis. When BCAA supplements are ingested, plasma BCAA levels rapidly increased, peaking at ~30 min after BCAA ingestion and thereafter gradually decreasing to normal levels in humans [2]. In this mechanism responsible for maintaining the low BCAA concentration levels, α-ketoisocaproate (a substrate of BCKDC) formed from leucine transamination acts as an inhibitor of BDK, resulting in the activation of BCKDC and then the promotion of BCAA catabolism [3].

3. Anabolic effects of BCAAs on protein metabolism

It has been demonstrated that mammalian target of rapamycin complex 1 (mTORC1), a highly conserved serine/threonine protein kinase, regulates protein metabolism in mammals; active mTORC1 promotes protein synthesis by activating the components involved in mRNA translation and inhibits protein degradation by suppressing autophagy [12]. Although it is known that many environmental signals regulate the mTORC1 pathway, amino acids, especially leucine, are potent activators of mTORC1 [13]. It has been recently reported that sestrin2 is the leucine sensor upstream of mTORC1; the binding of leucine to sestrin2 releases mTORC1 from negative regulation [13]. Because the liver has a low capacity to metabolize BCAAs (as described above) dietary leucine can directly affect the mTORC1 pathway by increasing the plasma leucine concentration. In this context, pharmacological supplementation with BCAAs is suggested to be a promising therapeutic strategy for liver cirrhotic patients with hypoalbuminemia [14].

4. Effects of BCAAs on glucose metabolism

Insulin plays an exclusive role in regulation of glucose metabolism, and obesity and type 2 diabetes induce hyperinsulinemia due to insulin resistance. Plasma BCAA concentrations are increased by 14-20% in patients with hyperinsulinemia under overnight fasting conditions [15] and are further increased by ingestion of dietary proteins [16,17]. Based on these findings and the results of animal study with high-fat diet and BCAA supplementation, it was proposed that BCAAs and their metabolites might contribute to insulin resistance [15]. Furthermore, it has been reported that 70 kDa ribosomal protein S6 kinase 1 (S6K1), downstream of mTORC1, negatively regulates insulin signaling under conditions of nutrient satiation by phosphorylation of insulin receptor substrate 1 (IRS1) serine residues involved in insulin resistance [18].

However, we found that hyperinsulinemia strongly decreases BCKDC activities in many tissues of diabetic rats by activation of BDK [19-21], thereby contributing to increased plasma BCAA concentrations. In contrast to rats with severe diabetic conditions such as Zucker diabetic fatty (ZDF) rats, who exhibit significantly higher plasma BCAA levels than normal rats, those in which diabetes is gradually developed, such as Otsuka Long-Evans Tokushima Fatty (OLETF) rats, showed normal plasma BCAA levels at the early stage of diabetic conditions with hyperinsulinemia [19], suggesting that an increase in plasma BCAA levels is not associated with hyperinsulinemia. Furthermore, chronic BCAA supplementation improved the deteriorated glucose tolerance in OLETF rats [19], CCl4-induced liver cirrhosis rats [22], and liver cirrhotic patients [14]. These findings suggest that BCAAs have therapeutic potential for the treatment of glucose intolerance, although further studies

are warranted to clarify the relationship between BCAA catabolism and insulin resistance.

5. Energy metabolism in muscle and BCAAs

It is thought that BCAA catabolism is initiated mainly in muscle tissues because of the high BCAT activity and large tissue mass in the body (as described above) suggesting the contribution of BCAAs to energy metabolism during exercise. It has been reported that chronic supplementation of an amino acid mixture with high (~60%) BCAA contents promoted mitochondrial biogenesis in heart and skeletal muscle of middle-aged mice, through increases in the gene expression of mitochondrial transcriptional regulators including peroxisome proliferator-activated receptor-γ coactivator-1α (PGC1α), resulting in an increase in the endurance capacity of the mice [23]. We also observed that chronic BCAA supplementation elevated the activities of mitochondrial marker enzymes (citrate synthase and cytochrome c oxidase) in skeletal muscle of sedentary mice (Xu, Kitaura, Shimomura, unpublished observation**)**.

In order to examine whether BCAAs have a role in adaptation to exercise training, we prepared muscle-specific BDK knockout (BDK-mKO) mice, in which free BCAA concentrations in muscle tissues are less than half of those in normal mice [24]. Two-week running exercise training (1 hour/day, 5 days/week) increased the endurance capacity more than 2 times compared to that before training in both normal and BDK-mKO mice; however, the endurance capacity after training was significantly (12%) less in BDK-mKO mice than normal mice. From the data obtained by the metabolome analysis of skeletal muscle of control and BDK-mKO mice, it was found in BDK-mKO mice that BCAAs were significantly catabolized with great production of

branched-chain acyl-carnitine, and that the levels of many components in the glycolytic pathway was significantly decreased in association with decreased levels of acetyl-CoA, citrate and isocitrate, but not succinate, fumarate or malate, suggesting perturbation of energy metabolism in the muscle of BDK-mKO mice. The reduced content of muscle glycogen was also found in BDK-mKO mice, which may be related with the perturbation of energy metabolism and low endurance capacity, because muscle glycogen content is an important factor determining endurance performance [25]. The accelerated BCAA catabolism may affect glycogen metabolism in the muscle [22]. These findings suggest that sufficient BCAAs are required during training for adequate adaptation of the animals to training [24].

6. Neurological development and BCAAs

Glutamate is an important excitatory neurotransmitter in the brain, and BCAAs (especially leucine) function to synthesize glutamate in astrocytes around neurons, since leucine enters the brain from the blood more rapidly than other amino acids and provides ~25% of all α-amino groups of glutamate [26]. Global BDK-KO (BDK-gKO) mice have been prepared and showed quite low levels of BCAAs in plasma and tissues including brain; the BCAA concentrations in the brains of BDK-gKO mice were ~30% of control mice. These animals showed neurological abnormalities as judged by their performance of hind limb flexion over the life span and epileptic seizures after 6-7 months of age [27], suggesting that BCAAs have an important role in neurological function. Subsequent to the preparation of BDK-gKO mice, patients with homozygous BDK mutations were identified, and it was found that these patients showed markedly low levels of plasma BCAAs and suffered from autism,

intellectual disability, and epilepsy [28, 29]. Since some of the neurological phenotypes in BDK-gKO mice were reversed by dietary BCAA supplementation, it may be possible to treat patients with BDK mutations with BCAA supplementation [28].

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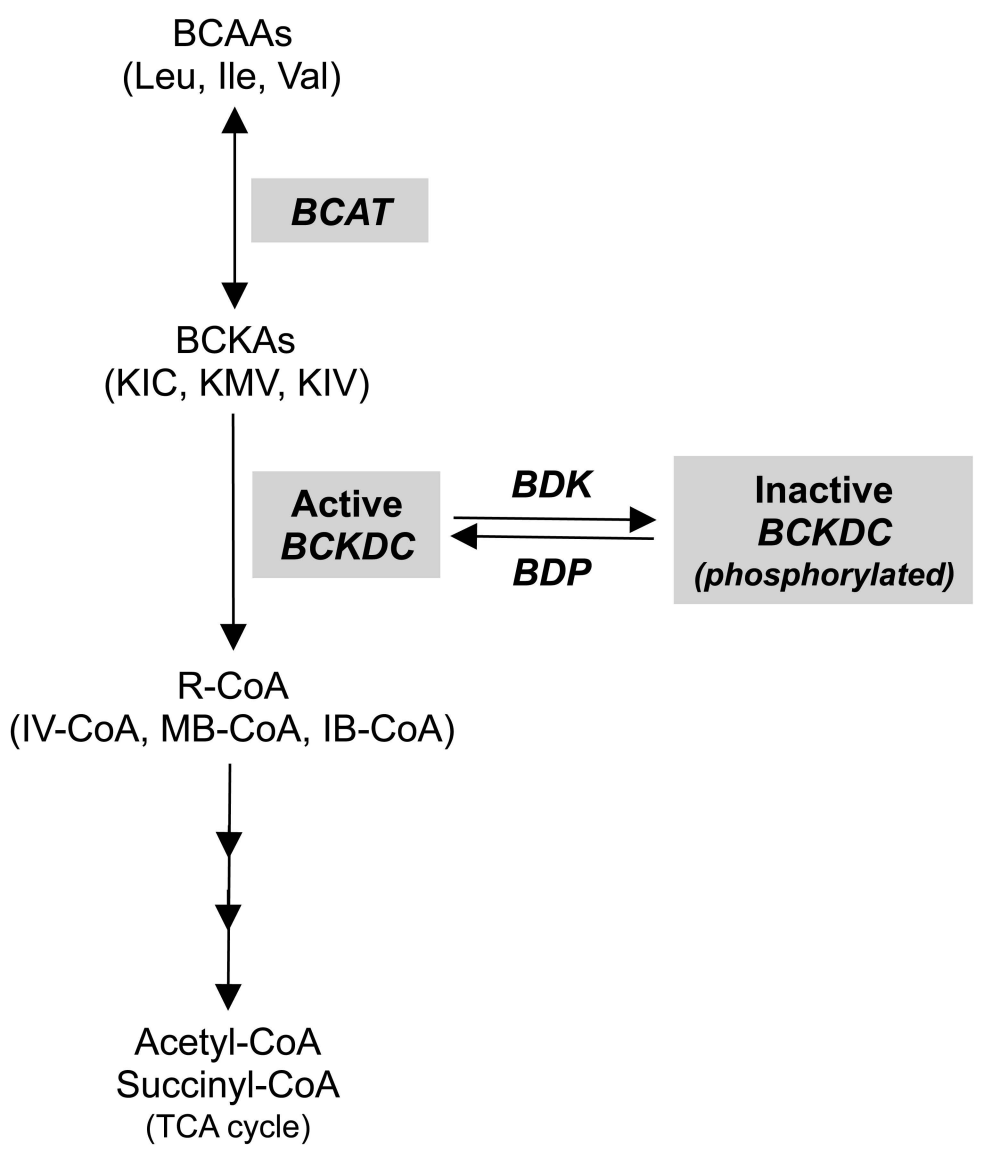
Figure legend

Figure 1. First two steps of BCAA catabolic pathway

BCAAs, branched-chain amino acids; BCAT, branched-chain aminotransferase; BCKAs, branched-chain α-keto acids; BCKDC, BCKA dehydrogenase (BCKDH) complex; BDK, BCKDH kinase; BDP, BCKDH phosphatase; KIC, α-ketoisocaproate; KMV, α-keto-ß-methylvalerate; KIV, α-ketoisovalerate; R-CoA, acyl-CoA; IV-CoA, isovaleryl-CoA; MB-CoA, α-methylbutyryl-CoA; and IB-CoA, isobutyryl-CoA.


BCAAs
(Leu, Ile, Val)
BCAT
BCKAs
(KIC, KMV, KIV)
Active
BCKDC
BDK
BDP
Inactive
BCKDC
(phosphorylated)
R-CoA
(IV-CoA, MB-CoA, IB-CoA)
Acetyl-CoA
Succinyl-CoA
(TCA cycle)