Note

Dissociation of Branched-Chain α-Keto Acid Dehydrogenase Kinase (BDK) from Branched-Chain α-Keto Acid Dehydrogenase Complex (BCKDC) by BDK Inhibitors

Taro Murakami, Masayuki Matsuo, Ayako Shimizu and Yoshiharu Shimomura*

Department of Material Science, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466–8555, Japan
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Summary Branched-chain α-keto acid dehydrogenase kinase (BDK) phosphorylates and inactivates the branched-chain α-keto acid dehydrogenase complex (BCKDC), which is the rate-limiting enzyme in the branched-chain amino acid catabolism. BDK has been believed to be bound to the BCKDC. However, recent our studies demonstrated that protein-protein interaction between BDK and BCKDC is one of the factors to regulate BDK activity. Furthermore, only the bound form of BDK appears to have its activity. In the present study, we examined effects of BDK inhibitors on the amount of BDK bound to the BCKDC using rat liver extracts. The bound form of BDK in the extracts of liver from low protein diet-fed rats was measured by an immunoprecipitation pull down assay with or without BDK inhibitors. Among the BDK inhibitors, α-ketoisocaproate, α-chloroisocaproate, and α-ketoisovalerate released the BDK from the complex. Furthermore, the release effect of these inhibitors on the BDK appeared to depend on their inhibition constants. On the other hand, clofibrac acid and thiamine pyrophosphate had no effect on the protein-protein interaction between two enzymes. These results suggest that the dissociation of the BDK from the BCKDC is one of the mechanisms responsible for the action of some inhibitors to BDK.

Key Words branched-chain α-keto acid dehydrogenase (BCKDC), branched-chain α-keto acid dehydrogenase kinase (BDK), protein-protein interaction, α-ketoisocaproate, α-chloroisocaproate

Branched-chain amino acids (BCAAs) are very abundant in our diets, making it necessary to have a high-capacity system for disposal of these amino acids to prevent the pathological consequences of maple syrup urine disease. On the other hand, tight control of BCAA disposal must be also maintained for the continuous availability of these essential amino acids for protein synthesis when BCAAs are insufficient in the diets. The BCAAs are special among the essential amino acids because of their ability to exert net anabolic and anti-proteolytic effects upon muscle tissues (1). For these reasons, elaborate mechanisms for control have evolved to regulate the activity for the branched-chain α-keto acid dehydrogenase complex (BCKDC), the enzyme most important in setting tissue levels of BCAAs.

The BCKDC catalyzes the oxidative decarboxylation of branched-chain α-keto acids and is the rate limiting, irreversible step of the catabolic pathways for leucine, isoleucine and valine (2). The complex consists of three components: specific dehydrogenase (E1) and transacylase (E2), and a specific dihydrolipoamide dehydrogenase (E3). Two regulatory enzymes are associated with the BCKDC: the specific kinase (BDK) (3, 4), which must be bound to the BCKDC for the activity, catalyzes the phosphorylation of two serine residues (Ser29s and Ser31s) of the E1α subunit, which completely inactivates the E1 component. Dephosphorylation of the BCKDC by the specific phosphatase (BDP) (5) is poorly characterized in terms of its structure and regulation.

Tight control of BCKDC activity is important for conserving as well as disposing of BCAAs, because BCAAs are required for synthesis of proteins, branched chain fatty acids, and neurotransmitters. Phosphorylation of the complex occurs when there is a need to conserve BCAAs for protein synthesis; dephosphorylation occurs when BCAAs are present in excess (reviewed in 2, 6). Activation of the complex can be achieved in the short term by inhibition of the activity of BDK by α-ketoisocaproate (KIC), the transamination product of leucine (7). Structural analogue of KIC including octanoate (8), α-chloroisocaproate (CIIC) (7), and clofibrac acid (CFIB) (9), also promote activation of the BCKDC by direct inhibition of the BDK.

Recent studies of ours have shown that protein-protein interaction between BDK and BCKDC may be important for regulating the BDK activity (10, 11). In these reports, a small amount of the BDK was bound to the BCKDC when BDK activity was low in rat muscle (11) and liver (10). On the other hand, the amount of

*To whom correspondence should be addressed.
E-mail: shimomura.yoshiharu@nitech.ac.jp

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Interaction between BDK and BCKDC

![Diagram](https://example.com/diagram.png)

**Fig. 1.** Dissociation of BDK from the BCKDC by BDK inhibitors. The liver extract was subjected to immunoprecipitation with polyclonal BCKDC E2 antiserum and protein A agarose overnight at 4°C. The BCKDC pulled down was then subjected to Western blot analysis with polyclonal BDK antiserum. The BDK inhibitors were added to the liver extract before immunoprecipitation at the following concentrations: KIC (µM): 0, 50, 100, and 200; CIIC (µM): 0, 25, 50, and 100; KIV (mM): 0, 1, 2.5, 2.5, and 5.0; Leu (mM): 0, 1.25, 2.5, and 5.0; CFIB (mM): 0, 0.5, 1.0, and 2.0; TPP (mM): 0, 0.25, 0.5, and 1.0. The bands represent the forms of BDK bound to the BCKDC after treatment with various concentrations of inhibitors described above.

The bound form of BDK was high when the tissue had high BDK activity (10, 11). These results suggested to us that the potent inhibitors of the BDK would inactivate the BDK by dissociating the BDK from the complex. In the present study, we have examined the effect of BDK inhibitors on the amount of the BDK bound to the BCKDC using rat liver extracts.

Rat liver extract was prepared from rats fed with an 8% protein diet (12) as reported previously (13). The liver extract was known to have a large amount of the bound form of BDK (10). The actual (before activation) and total (after full dephosphorylation and activation by exogenous lambda protein phosphatase) activities (mean ± SE for four rats) were 0.01 ± 0.01 and 0.46 ± 0.03 µmol/min/g tissue, respectively, indicating that the complex was fully inactivated in vivo. These values are consistent with those reported previously (12). The liver extract (250 µg of protein) was subjected to immunoprecipitation with polyclonal BCKDC E2 antiserum and protein A agarose overnight at 4°C as described previously (10). In this experiment, the BDK inhibitors were added to the liver extract, which was used for immunoprecipitation of the BCKDC. The BCKDC which was pulled down by the immunoprecipitation was then subjected to Western blot analysis with polyclonal BDK antiserum (10).

It has been believed that KIC is an important physiological regulator (inhibitor) of BDK in the short-term regulation (17, 8) and reported that the IC50 of KIC for the BDK was 65 µM. The amount of the bound form of BDK was decreased by KIC in a dose-dependent manner (Fig. 1). CIIC, which is a structural analogue of KIC and has almost the same IC50 value (80 µM) as that of KIC, was also examined. As expected, CIIC released the BDK from the BCKDC in a dose-dependent manner as observed by KIC (Fig. 1). Since α-ketoisovalerate (KIV) has higher IC50 value (2.5 mM) than that of KIC or CIIC, we examined the effect of KIV at a higher range of the concentration than that of KIC. KIV also released the BDK from the complex; however, the effect was much lower than that of KIC and its analogue (Fig. 1). Because leucine is a precursor of KIC, we examined its effect of releasing the BDK from the BCKDC. However, the BDK was not released from the BCKDC up to 5 mM (Fig. 1). These results indicate that the effects of various inhibitors on the release of BDK from the BCKDC are dependent on the inhibition constants.

CFIB is best known for its ability to lower the blood lipid level (14) and to increase hepatic peroxisomes (15). CFIB is also known to attenuate BDK activity by direct inhibition (9) and by decreasing its expression (16). The IC50 of CFIB has been reported at 0.33 mM (9). So we hypothesized that CFIB would have the effect of releasing the BDK from the complex. However, CFIB did not release the BDK from the complex up to 2 mM (Fig. 1), suggesting that CFIB does not affect the protein-protein interaction between BDK and the BCKDC. The thiamine pyrophosphate (TPP) is also known as one of the potent inhibitors (Kd=4.0 µM) of BDK (17). However, TPP had no effect on the amount of BDK bound to the complex up to 1 mM (Fig. 1).

Although limited numbers of the inhibitors were examined in this study, our results indicate that at least two types of BDK inhibitors exist; one of them interferes...
with the protein-protein interaction between BDK and BCKDC, resulting in inhibition of the BDK activity. This type of inhibitors involves the substrates of the BCKDC or compounds having a structure quite similar to the substrates. Another type has no effect on the interaction between two proteins. These findings indicate that interaction between BDK and the BCKDC is important in regulation of the BDK activity. This supports our concept (10,11) reported previously that the binding of the BDK to the complex is one of the mechanisms responsible for regulation of the kinase activity. However, the results obtained in the present study also indicate a possibility that regulation of the kinase activity occurs without dissociation of two enzymes. Further studies are required to establish the mechanisms by which BDK inhibitors affect the protein-protein interaction between BDK and BCKDC. We are pursuing.

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