Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimica et Biophysica Acta 1647 (2003) 61–65
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.bba-direct.com

Review

Human mitochondrial branched chain aminotransferase: structural basis for substrate specificity and role of redox active cysteines

Myra E. Conway[a], Neela Yennawar[b], Reidar Wallin[c], Leslie B. Poole[a], Susan M. Hutson[a,*]

[a] Department of Biochemistry, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA
[b] Department of Biochemistry and Molecular Biology, Althouse Lab, Pennsylvania State University, University Park, PA 16802, USA
[c] Department of Internal Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA

Received 14 July 2002; accepted 11 September 2002

Abstract

Crystal structures of the fold type IV pyridoxal phosphate (PLP)-dependent human mitochondrial branched chain aminotransferase (hBCATm) reaction intermediates have provided a structural explanation for the kinetically determined substrate specificity of hBCATm. The isoleucine side chain in the ketimine intermediate occupies a hydrophobic binding pocket that can be defined by three surfaces. Modeling of amino acids on the ketimine structure shows that the side chains of nonsubstrate amino acids such as the aromatic amino acids, alanine, or aspartate either are unable to interact through van der Waals' interactions or have steric clashes. The structural and biochemical basis for the sensitivity of the mammalian BCAT to reducing agents has also been elucidated. Two cysteine residues in hBCATm, Cys315 and Cys318 (CXXC), are part of a redox-controlled mechanism that can regulate hBCATm activity. The residues surrounding Cys315 and Cys318 show considerable sequence conservation in the prokaryotic and eukaryotic BCAT sequences, however, the CXXC motif is found only in the mammalian proteins. The results suggest that the BCAT enzymes may join the list of enzymes that can be regulated by redox status.

Keywords: Aminotransferase; Branched chain amino acid; Human; Mitochondrion; Structure; Redox-active

1. Introduction

Branched chain aminotransferases (BCAT) (EC 2.6.1.42) catalyze the transamination of the branched chain amino acids, leucine, isoleucine, and valine, to their respective α-keto acids, α-ketoisocaproate, α-keto-β-methylvalerate, and α-ketoisovalerate. Bacteria appear to contain only a single BCAT isoform [1]. However, in humans (and other mammals) there are two BCAT isozymes, mitochondrial (hBCATm) and cytosolic (hBCATc) [2]. The mammalian isozymes are homodimers and show tissue-specific expression [3–5].

Most pyridoxal phosphate (PLP)-dependent aminotransferases have been placed in the fold type I or L-aspartate aminotransferase family. The BCAT are an exception as they are in the fold type IV family of the PLP-dependent enzymes and are the only mammalian proteins in this group [5–7]. A unique feature of this family is that the proton is added to or abstracted from the C4′ atom of the coenzyme-imine or external aldimine on the *re* face instead of the *si* face of the PLP cofactor [8]. Bacterial D-amino acid aminotransferase [6] and bacterial enzyme 4-amino-4-deoxychorismate lyase (a β-lyase) are also members of the fold type IV family [9], and we have recently found that the human BCAT isozymes can also catalyze an effective β-lyase reaction [10].

A unique property of BCAT isozymes is their sensitivity to reducing agents, whereby a loss of activity occurs unless BCATm or BCATc are stored in a reducing environment [3,11,12]. Sulfhydryl reagents inhibit both BCAT isozymes. Human BCATm contains six cysteine residues [3]. Titration of two of these thiol groups leads to almost complete inhibition of activity [3]. The two reactive cysteines have been identified and have been shown to have redox activity.

Abbreviations: BCAT, branched chain aminotransferase; hBCATm, human mitochondrial branched chain aminotransferase; hBCATc, human cytosolic branched chain aminotransferase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; NEM, *N*-ethylmaleimide; PLP, pyridoxal phosphate

* Corresponding author. Tel.: +1-336-716-6055; fax: +1-336-716-7671.
E-mail address: shutson@wfubmc.edu (S.M. Hutson).

1570-9639/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S1570-9639(03)00051-7

This review describes recent studies that have provided insight into the structure of the BCAT and role of cysteine residues in regulating enzyme activity.

2. Results and discussion

2.1. Structure and substrate specificity

The crystal structures of hBCATm in the PLP (PDB codes 1EKP, 1EKF), ketimine (1KT8), and PMP (1KTA) forms have been elucidated [5,7]. The catalytically competent structure of hBCATm is a dimer [3]. The three-dimensional structures of hBCATm show that each monomer is composed of a small domain (1–170) and a large domain (182–365) connected by an interdomain loop that is composed of 11 amino acids [7].

These structures have provided information on the molecular basis for the known substrate specificity of the mitochondrial isozyme hBCATm. The kinetic characterization of hBCATm has shown that branched chain amino acids, their straight chain analogs, and glutamate are substrates; however, the aromatic amino acids, aspartate, or alanine are not substrates [3]. The ketimine form of hBCATm was obtained by soaking crystals in the PLP form with isoleucine [7]. Based on the position of the isoleucine side chain, models of other side chains were built [7]. Three surfaces form the side chain binding pocket (Fig. 1). Phe75, Tyr207 and Thr240 form surface A and Phe30, Tyr141 plus Ala314 form Surface B. Tyr70*, Leu153* and Val155* (asterisk denotes residue from the opposite subunit) form surface C. Branched chain amino acids fit into the substrate pocket enclosed by A, B and C and have van der Waals' interactions with A or B, whereas the bulky phenylalanine side chain would have steric clashes with A or B in either of its minimum energy conformations (Fig. 1A). The small alanine side chain would fall short of interacting through van der Waals' contacts with any of the surfaces. The side chain carboxyl group of the substrate glutamate can form good hydrogen bond interactions with the side chains of Tyr141 of surface B, Tyr70* of surface C and Arg143 (Fig. 1B). However, the side chain carboxyl group of aspartate would fall short of forming these hydrogen bonds.

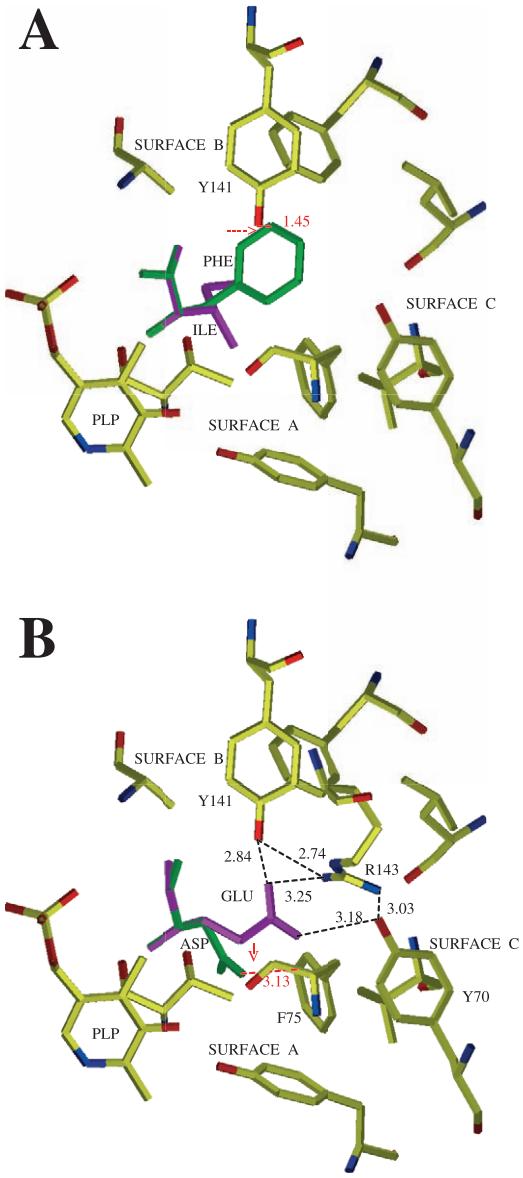


Fig. 1. Diagram of Phe, Glu, and Asp modeled into the hBCATm substrate side chain binding pocket. Models were based on the position of the isoleucine side chain in the hBCATm ketimine structure. (A) Phe and Ile. The steric clash between Phe and Tyr141 of surface B is shown by the red arrow and dashed line. (B) Asp and Glu. The Glu the side chain carboxyl group shows favorable hydrogen bond interactions (black dashed lines) with Tyr141 (surface B), Arg143, and Tyr70* (surface C). The steric clash between Asp and Phe75 of surface A is shown by the red arrow and dashed line. Adapted from Ref. [7].

2.2. Domain closure

In bacterial *Escherichia coli* BCAT, when a substrate analogue is present in the substrate binding pocket, the active site is closed by movement of the interdomain loop [13]. In contrast, in hBCATm, binding of the substrate involves movement of the interdomain loop such that it increases access to the active site (Fig. 2). This is enabled by movement of the ring atom of Tyr173 which is part of the interdomain loop. Thus instead of domain closure as is classically seen in AspAT or closure by movement of the interdomain loop observed in the *E. coli* BCAT, in hBCATm, the conformation of Tyr173 changes dramatically in the various forms of the enzyme and may control entry or exit of substrates and products (Fig. 2).

2.3. Role of cysteine residues in hBCATm and identification of a redox active CXXC center

Human BCATm contains six cysteine residues per monomer, and the enzyme requires a reducing environment to maintain maximal catalytic activity. Labeling of hBCATm

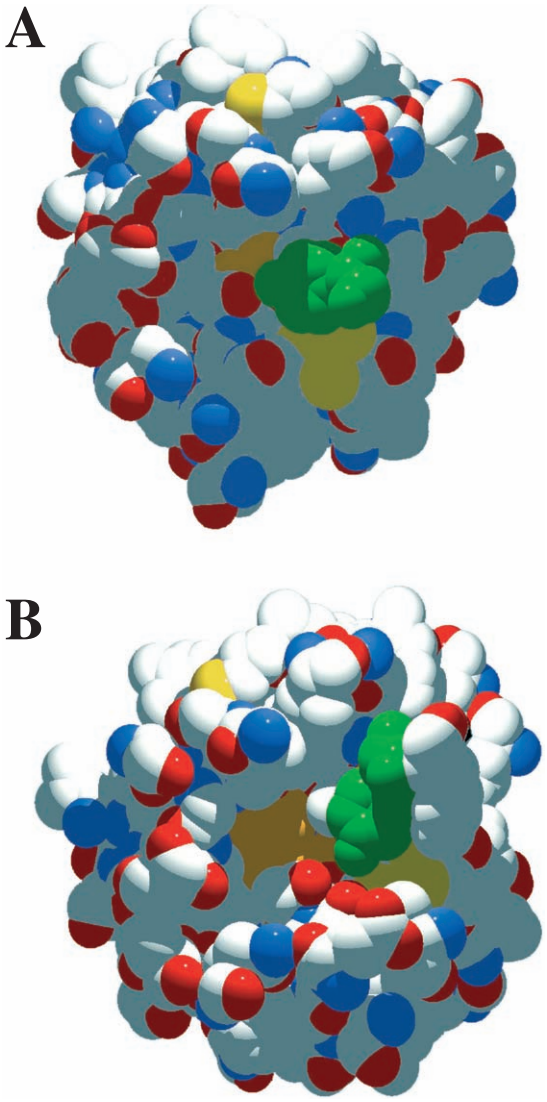


Fig. 2. Space-filling diagrams showing the active site of hBCATm monomer B in the (A) PLP and (B) ketimine form. The side chain of Tyr173 ring (in green) is partially blocking the active site in the hBCATm PLP form. In the ketimine form, loop 171–181 moves, the conformation of Tyr173 changes, and the active site remains open. Adapted from Ref. [7].

by sulfhydryl reagents showed that the enzyme is inhibited by a wide variety of thiol reagents [3,11,12,14]. Labeling of two thiol groups with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (reversible modifier of thiol groups) or *N*-ethylmaleimide (NEM) (irreversible modifier of thiol groups) correlated with a loss of hBCATm activity [14]. The reactive cysteine residues were identified as Cys315 and Cys318 after labeling with ^{125}I β-(4-hydroxyphenyl)ethylmalemide and subsequent chemical cleavage with cyanogen bromide [14].

Cys315 and Cys318 are located about 10 Å from the active site in a beta-turn. This turn is preceded by another beta-turn and followed by a beta-sheet, a feature that is seen in the structures of *E. coli* BCAT and D-amino acid aminotransferase [7,13,15]. Residues surrounding these cysteines are highly conserved in all BCATs, however, the CXXC motif

Table 1
Alignment of the amino acid sequence of hBCAT with eukaryotic and prokaryotic organisms[a]

BCAT PROTEINS	SEQUENCE ALIGNMENTS
hBCATc	RVREVFGSGTA**CVVC**PVHRILYK
hBCATm	RVREVFGSGTA**CQVC**PVHRILYK
rBCATc	RVREVFGSGTA**CVVC**PVHQILYE
rBCATm	RVREVFGSGTA**CQVC**PVHQILYE
sBCATc	RVREVFGSGTA**CVVC**PVHQILYQ
sBCATm	RVREVFGSGTA**CQVC**PVHQILYQ
C.elegans 1	RLYEMFGSGTACVVSPVGKILYH
C.elegans 2	RVHEFFVSGTAANVGPVSEIVYC
A.thaliana	DADEVFCTGTAVVVAPVGTITYQ
E.coli	LADEVFMSGTAAEITPVRSVDGI
M.tuber	EITEVFACGTAAVITPVARVRHG
	* * *** **

[a] The CXXC region is highlighted in bold and is conserved only in mammalian BCAT proteins. The residues highlighted by an asterisk are conserved in nearly all the fold type IV protein sequences. Accession numbers and sources of the putative BCAT proteins shown are: hBCATc [3]; hBCATm (human, U21551); rBCATc (rat, NM017253); rBCATm (rat, U68417); sBCATc (sheep, AF184916); sBCATm (sheep, AF050173); *Caenorhabditis elegans* 1 (P54688); *Caenorhabditis elegans* 2 (NM075613); *Arabidopsis thaliana* (AJ271731); *Escherichia coli* (NC000913); *Mycobacterium tuberculosis* (AE007072) From Conway et al. [14], reprinted with permission.

is found only in the mammalian BCATs (Table 1) [14]. The conserved residues Gly312, Thr313 and Ala314 (in the beta-turn) align to form a net positive dipole moment in the region of the PLP oxygens, while Thr313 also interacts with the substrate α-carboxyl group (Fig. 3) [7]. Therefore, although Cys315 and Cys318 do not appear to interact directly with the PLP cofactor or substrate, they are structurally important [7]. The crystal structures also show that Cys315 and Cys318 share a hydrogen bond, which under oxidizing conditions,

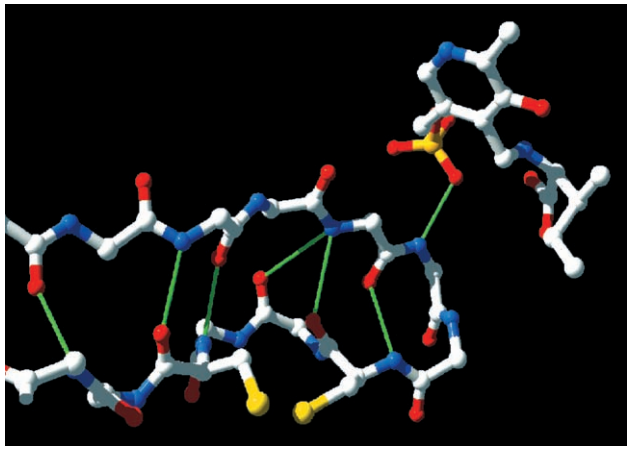
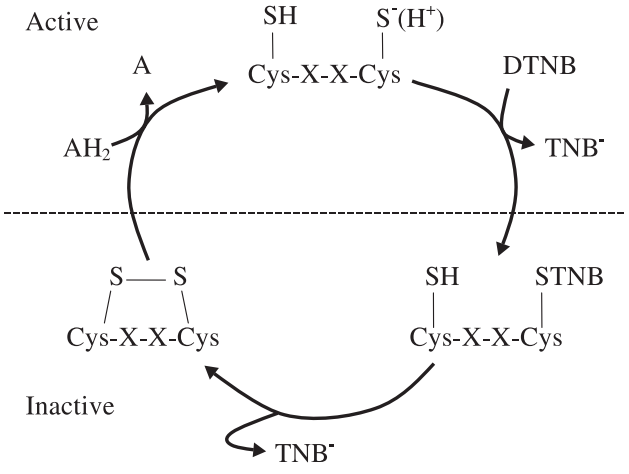


Fig. 3. The location of Cys315 and Cys318 in relation to the peptide dipole interaction with the phosphate of the cofactor in the PLP (A). Cys315 and Cys318 participate in a thiol–thiolate hydrogen bond with the average distance between the sulfurs ranging from 3.09 to 3.46 Å in the different crystal forms. The peptide dipole vectors in the beta-turn including residues 312–314 are parallel to each other such that their partial positively charged end faces the negatively charged phosphates. From Conway et al. [14], reprinted with permission.



Scheme 1.

could reasonably form a disulfide bond. This thiol–thiolate interaction is also seen in redox-active disulfide centers of both reduced thioredoxin [16] and the N-terminal domain of AhpF [17], a flavin containing disulfide reductase.

Initial evidence that the CXXC region in hBCATm is sensitive to redox resulted from titration studies of hBCATm with DTNB. Spectrophotometric titrations indicated that incubation of hBCATm with DTNB resulted in labeling of the protein with two TNB anions. However, subsequent analysis of the DTNB-labeled protein using electrospray ionization mass spectrometry showed that there was actually a mixture of species rather than the expected single hBCATm protein peak containing two TNB molecules [14]. As shown in Scheme 1, after formation of a TNB-conjugated cysteine, the second thiol group in hBCATm can react to form an intrasubunit disulfide bond releasing a second TNB anion. The net result is the reduction of one DTNB molecule to form two free TNB anions and oxidation of the protein dithiol to the disulfide. This hypothesis is consistent with the mass spectrometry analysis showing that DTNB-labeled hBCATm is predominantly a mixture of an oxidized form containing a disulfide bond and singly TNB-labeled protein [14].

The presence of an active CXXC center in hBCATm raised the possibility that redox status may regulate BCAT activity. To test this hypothesis, hBCATm was treated with increasing concentrations of H_2O_2. The results showed that H_2O_2 resulted in a "dose-dependent" decrease in titratable thiols and a corresponding, almost complete loss in activity that was reversible on addition of DTT [14]. Furthermore, at physiological temperature, oxidation and reversible inactivation of hBCATm by H_2O_2, is rapid, occurring within 1 min (unpublished data). These results suggest that Cys315 and Cys318 act as a redox-active CXXC center that can regulate hBCATm activity.

2.4. Future directions

There is considerable evidence that regulation of the redox status of cysteine residues in cellular proteins controls their activity and function [18]. In mammalian cells, cellular H_2O_2 can oxidize methionine and cysteine residues in proteins to methionine sulfoxide and cysteine sulfenic acid (or disulfide), respectively, in response to extracellular stimuli including cytokines and growth factors [19]. For example, direct inhibitory effects of H_2O_2-mediated oxidation that result in functional changes of protein tyrosine phosphatases and the protease papain have been reported [20]. Moreover, it has been suggested that peroxide induces reversible glutathionylation of α-ketoglutarate dehydrogenase in heart mitochondria [21]. Thus, it is our hypothesis that redox state may play a role in regulation of mammalian BCAT activity or proteins that interact with the BCAT. For example, we have evidence that hBCATm can associate with the second enzyme in the branched chain amino acid catabolic pathway and that binding of hBCATm to this multienzyme complex, the branched chain α-keto acid dehydrogenase enzyme complex, is influenced by NADH (unpublished data).

Furthermore, the discovery of two additional forms of hBCATm, increases the possibility that hBCATm is a multifunctional enzyme. An alternatively spliced form of hBCATm that contains a 12 amino acid deletion that starts immediately following Cys315 (lacks CXXC) has been reported to bind to the β1 form of the thyroid receptor and act as a co-repressor [22]. A second alternatively spliced form of hBCATm with unknown function has also been reported [23]. This protein lacks 100 amino acids in the N-terminal part of the protein, i.e., lacks the mitochondrial targeting sequence, and is found in the cytosol of most human tissues. This form of hBCATm retains the CXXC center but lacks two residues forming surface B (Phe30) and C (Tyr70*) of the substrate side chain binding pocket. Preliminary experiments suggest that this protein does not have BCAT activity (unpublished data). These exciting new developments may lead to identification of new functions for the mammalian BCAT family (BCATm and BCATc), as has been described for a number of other metabolic enzymes.

Acknowledgements

This study was supported by a grant from the National Institute of Health to S.M.H. (RO1 DK34738) and an Established Investigatorship from the American Heart Association to L.B.P.

References

[1] S. Kamitori, Y. Odagaki, K. Inoue, S. Kuramitsu, H. Kagamiyama, Y. Matsuura, T. Higuchi, J. Biochem. 105 (1988) 671–672.
[2] S.M. Hutson, D. Fenstermacher, C. Mahar, J. Biol. Chem. 263 (1988) 3618–3625.
[3] J. Davoodi, P.M. Drown, R.K. Bledsoe, R. Wallin, G.D. Reinhart, S.M. Hutson, J. Biol. Chem. 273 (1998) 4982–4989.

[4] K. Okada, K. Hirotsu, M. Sato, H. Hayashi, H. Kagamiyama, J. Biochem. 121 (1997) 637–641.
[5] N. Yennawar, J. Dunbar, M. Conway, S. Hutson, G. Farber, Acta Crystallogr., D Biol. Crystallogr. 57 (2001) 506–515.
[6] N.V. Grishin, M.A. Phillips, E.J. Goldsmith, Protein Sci. 4 (1995) 1291–1304.
[7] N. Yennawar, M.E. Conway, H.P. Yennawar, G.K. Farber, S.M. Hutson, Biochemistry 41 (39) (2002) 11592–11601.
[8] T. Yoshimura, K. Nishimura, J. Ito, N. Esaki, H. Kagamiyama, J.M. Manning, K. Soda, J. Am. Chem. Soc. 115 (1993) 3897–3900.
[9] T. Nakai, H. Mizutani, I. Miyahara, K. Hirotsu, S. Takeda, K.-H. Jhee, T. Yoshimura, N. Easki, J. Biochem. (Tokyo.) 128 (2000) 29–38.
[10] A.L. Cooper, S.A. Bruschi, M. Conway, S.M. Hutson, Biochem. Pharmacol. 65 (2) (2002) 181–192.
[11] R. Wallin, T.R. Hall, S.M. Hutson, J. Biol. Chem. 265 (1990) 6019–6024.
[12] T.R. Hall, R. Wallin, G.D. Reinhart, S.M. Hutson, J. Biol. Chem. 268 (1993) 3092–3098.
[13] K. Okada, K. Hirotsu, H. Hayashi, H. Kagamiyama, Biochemistry 40 (2001) 7453–7463.
[14] M.E. Conway, N. Yennawar, R. Wallin, L.B. Poole, S.M. Hutson, Biochemistry (2002).
[15] S. Sugio, G.A. Petsko, J.M. Manning, K. Soda, D. Ringe, Biochemistry 34 (1995) 9661–9669.
[16] L.A. Tartaglia, G. Storz, M.H. Brodsky, A. Lai, B.N. Ames, J. Biol. Chem. 265 (1990) 10535–10540.
[17] Z.A. Wood, L.B. Poole, P.A. Karplus, Biochemistry 40 (2001) 3900–3911.
[18] A. Claiborne, T.C. Mallett, J.I. Yeh, J. Luba, D. Parsonage, Adv. Protein Chem. 58 (2001) 215–276.
[19] H.M. Lander, FASEB J. 11 (1997) 118–124.
[20] W. Yalin, K. Ki-Sun, S.G. Rhee, FEBS Lett. 440 (1998) 111–115.
[21] A.C. Nulton-Persson, L.I. Szweda, XIth Meeting of the Society for Free Radical Research International (2002).
[22] H.M. Lin, M. Kaneshige, L. Zhao, X. Zhang, J.A. Hanover, S.Y. Cheng, J. Biol. Chem. 51 (2001) 48196–48205.
[23] N.G. Than, B. Sumegi, G.N. Than, Sz. Bellyei, H. Bohn, Placenta 22 (2001) 235–243.