Crystal Structures of Complexes of the Branched-Chain Aminotransferase from *Deinococcus radiodurans* with α -Ketoisocaproate and L-Glutamate Suggest the Radiation Resistance of This Enzyme for Catalysis

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Branched-chain aminotransferases (BCAT), which utilize pyridoxal 5'-phosphate (PLP) as a cofactor, reversibly catalyze the transfer of the α -amino groups of three of the most hydrophobic branched-chain amino acids (BCAA), leucine, isoleucine, and valine, to α -ketoglutarate to form the respective branched-chain α -keto acids and glutamate. The BCAT from *Deinococcus radiodurans* (DrBCAT), an extremophile, was cloned and expressed in *Escherichia coli* for structure and functional studies. The crystal structures of the native DrBCAT with PLP and its complexes with L-glutamate and α -ketoisocaproate (KIC), respectively, have been determined. The DrBCAT monomer, comprising 358 amino acids, contains large and small domains connected with an interdomain loop. The cofactor PLP is located at the bottom of the active site pocket between two domains and near the dimer interface. The substrate (L-glutamate or KIC) is bound with key residues through interactions of the hydrogen bond and the salt bridge near PLP inside the active site pocket. Mutations of some interaction residues, such as Tyr71, Arg145, and Lys202, result in loss of the specific activity of the enzymes. In the interdomain loop, a dynamic loop (Gly173 to Gly179) clearly exhibits open and close conformations in structures of DrBCAT without and with substrates, respectively. DrBCAT shows the highest specific activity both in nature and under ionizing radiation, but with lower thermal stability above 60°C, than either BCAT from *Escherichia coli* (eBCAT) or from *Thermus thermophilus* (HB8BCAT). The dimeric molecular packing and the distribution of cysteine residues at the active site and the molecular surface might explain the resistance to radiation but small thermal stability of DrBCAT.

Branched-chain aminotransferase (BCAT), which contains pyridoxal 5'-phosphate (PLP) as a cofactor, is the key enzyme in the biosynthetic path of hydrophobic amino acids, e.g., leucine, isoleucine, and valine, to α -ketoglutarate to form the respective branched-chain α -keto acids and glutamate (27, 53). BCAT enzymes are distributed widely in many species (10, 28), from bacteria containing a single BCAT enzyme (33) to mammals with these enzymes in both mitochondrial and cytosolic forms (24, 26). In plants, BCAT might be restrained by hormone treatment, resulting in the regulation of endogenous hormones by affecting specific genes (18). Among four classified families with PLP-dependent enzymes of varied fold types based on similarities in the sequence, secondary structure, and hydrophobicity profiles, BCAT belongs to the family type IV that transfers protons on the *re*-face of the PLP cofactor (30, 60). BCAT can be used to synthesize L-tert-leucine and other branched-chain or unnatural amino acids from their respective keto acids; they have a broad applicability in the synthesis of fine chemicals and pharmaceuticals (52). The enzyme functions via a ping-pong kinetic mechanism that comprises two half reactions, each with three main steps, shown as follows (34).

L-leucine + α -ketoglutarate

$\leftrightarrow$ α -ketoisocaproate (KIC) + L-glutamate

L-valine + α -ketoglutarate

$\leftrightarrow$ α -ketoisovalerate (KIV) + L-glutamate

L-isoleucine + α -ketoglutarate

$\leftrightarrow$ α -keto- β -methylvalerate (KMV) + L-glutamate

Deinococcus radiodurans R1 is a Gram-positive, extremophile, red-pigmented, nonmotile bacterium that is well-known for its extreme resistance to the lethal effects of ionizing radiation, UV radiation, and hydrogen peroxide (40, 56). *D. radiodurans* has been described as the organism most resistant to radiation: exponentially growing cells were 200 times as resistant to ionizing radiation and 20 times as resistant to UV irradiation as *Escherichia coli* (56). It was originally identified in irradiated canned meat and has been found widely from environments rich in organic nutrients, including soil, animal feces, and processed meats, and from dry, nutrient-poor environments, including weathered granite in a dry Antarctic valley, room dust, and irradiated medical instruments (38). The complete genome sequence of *D. radiodurans* R1 has been determined (37, 56); it reveals the related chromosomes

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FIG 1 Ping-pong reaction mechanism of BCAT.

and megaplasmid of a bacterium able to survive under conditions of starvation, oxidative stress, and extensive DNA damage. The mechanism of repair of DNA double-strand breaks was studied intensively (16, 17, 36, 39). The protein components of *D. radiodurans* were valuable sources for the investigation of structure-function relations possibly due to their unique structures and amino acid compositions that might confer special properties, such as thermal stability or radiation resistance.

DrBCAT (BCAT from *D. radiodurans*), comprising 358 amino acids, contains PLP as a cofactor for its function. The enzyme was expected to have particular activity and stability in an environment of ionizing radiation in terms of the three-dimensional (3-D) structure, amino acid sequence, and molecular assembly. Several structures of BCAT from various species were previously determined, including human mitochondrial BCAT (hBCATm) (59) and human cytosolic BCAT (hBCATc) from human source (23), BCAT from *E. coli* (eBCAT) (42), HB8BCAT from *Thermus thermophilus* (PDB accession no. 1WRV [unpublished work]), BCAT from *Mycobacterium tuberculosis* (MtBCAT) (6), and BCAT from *Mycobacterium smegmatis* (MsBCAT) (6). The hBCATm is expressed in body tissues, whereas hBCATc was localized primarily in the central nervous system (11, 25, 61). The mammalian BCATs were commonly found as dimers with molecular masses of 41.3 and 43 kDa of the hBCATm and hBCATc monomers, respectively. eBCAT and HB8BCAT were hexamers with molecular masses of ~34 kDa for each monomer, whereas both MtBCAT and MsBCAT were dimers with similar molecular masses of 40 kDa for each monomer. Among these structures, DrBCAT shows fairly small sequence identities of 32.0, 33.0, 32.5, 29.1, 42.6, and 42.6% in comparison with hBCATm, hBCATc, eBCAT, HB8BCAT, MtBCAT, and MsBCAT, respectively. Moreover, the numbers of cysteine (Cys) residues in DrBCAT (6 [the number of Cys residues shown in parentheses after the enzyme]), hBCATm (6), hBCATc (10), eBCAT (3), HB8BCAT (1), MtBCAT (3), and MsBCAT (2) vary significantly with the distribution in the corresponding sequences.

In this work, we have expressed and purified the DrBCAT to study the activity assay and structure, which is the first reported BCAT from a radiation-resistant organism. We describe the crystal structures of DrBCAT and its complexes with α-ketoisocaproate and L-glutamate, for the reaction shown in Fig. 1.

We surmise that a comparison of amino acid sequences, structures, and enzymatic activities among those BACT enzymes from various species might provide a structural basis for a radiation-resistant mechanism and a specific activity of DrBCAT greater than those of eBCAT and HB8BCAT.

MATERIALS AND METHODS

Protein purification. The details of protein purification of DrBCAT were described (8). In brief, the pQE30 expression vector (Qiagen) containing the DrBCAT cDNA was transformed into *E. coli* JM109 cells for overexpression in Luria-Bertani (LB) broth (200 ml) containing ampicillin (50 μg/ml) for incubation overnight at 37°C. Isopropyl-β-D-thiogalactopyra-

nose (IPTG) (final concentration of 1 mM) was added to the culture for induction at 20°C for 18 h before the cells were harvested on centrifugation (6,800 × g). The medium was discarded, and the cell pellet was resuspended with binding buffer (250 ml) containing NaCl (0.5 M), imidazole (5 mM), and Tris-HCl (20 mM; pH 7.9), and subjected to cell disruption by ultrasonication and a high-pressure homogenizer. The suspension was collected on centrifugation (24,000 × g) at 4°C for 30 min. The soluble protein extract was passed through a His-tag Ni²⁺-nitrilotriacetic acid (NTA) column twice to increase its homogeneity. The protein was concentrated on Amicon (molecular weight cutoff [MWCO] of 10,000; Millipore), and the buffer was concurrently changed to Tris-HCl buffer (20 mM; pH 7.0).

Activity assay. BCAT catalyzes the process from branched-chain 2-oxo acid-dependent transamination of L-glutamate that is coupled to L-leucine and 2-oxoglutarate. The process is further catalyzed by L-aspartate aminotransferase (AspAT) (EC 2.6.1.1) from 2-oxoglutarate to oxaloacetate. L-Malate dehydrogenase (MDH) (EC 1.1.1.37) was used to catalyze the reaction from oxaloacetate to L-malate through oxidation of NADH to NAD⁺ as the indicator system (49). The specific measurement of the decrease of NADH absorbance at 340 nm can monitor the rate of transamination that can transform to become the activity of the enzyme. The BCAT activity was determined by monitoring the temporal course of NADH consumption at 340 nm. The amounts of NADH change were recorded every minute with a spectrophotometer (model U-2010; Hitachi). A thermostated cuvette holder (model B401D; First Scientific) was used to control the reaction temperature at 25°C. The standard assay conditions as given in Table 1 were applied. The enzyme activity concentration in the assays is limited to ≤25 U/liter (1 unit [U] is 1 μmol of oxaloacetate per unit volume and unit time).

The assay volume is 1 ml; the molar extinction coefficient is $\epsilon_M = 6,225 \text{ M}^{-1} \text{ cm}^{-1}$. According to the Beer-Lambert law, the enzyme activity was measured under the standard conditions given above.

$$\text{Specific activity} = (\Delta A \times 10^6) / (6,225 \times V \times C)$$

where ΔA is the change in absorbance, V denotes the assay volume of BCAT (in milliliters), C is the concentration of BCAT (in milligrams per milliliter), and specific activity is given in units per milligram where the unit is the activity unit (1 μmol of oxaloacetate per min⁻¹).

Crystallization and X-ray data collection. The crystals of native DrBCAT were obtained with the hanging-drop vapor diffusion method at a temperature of 20°C. The protein solution comprised DrBCAT (14 mg ml⁻¹) with PLP (10 μM) in Tris-HCl buffer (0.1 M; pH 7.0). Crystallization drops were prepared on mixing the protein solution (1 μl) with an equal volume of reservoir solution (100 μl) containing magnesium chloride (0.15 M), polyethylene glycol 8000 (15% [wt/vol]) in Tris-HCl buffer

TABLE 1 Standard assay conditions for spectrophotometric determination of BCAT activity

Component or enzyme	Component concn (mM) or enzyme activity in assay (μmol × min ⁻¹ × liter ⁻¹)
Components	
Tris-HCl (pH 8.3)	100
KIC	2.1
L-Glutamate	300
NADH	0.2
PLP	0.1
L-Aspartate	200
Enzymes	
L-Aspartate aminotransferase	2,500
L-Malate dehydrogenase	5,000
BCAT	Variable but ≤25

(0.1 M; pH 7.4). The crystals of native *DrBCAT* with PLP were grown to the terminal size of 0.6 by 0.02 by 0.02 mm³ after 2 weeks.

Cocrystals of *DrBCAT*-KIC and *DrBCAT*-L-glutamate were also obtained with a crystallization protocol similar to that of native crystals. *DrBCAT* (14 mg ml⁻¹) with α -ketoisocaproate (KIC) (10 mM), referred as *DrBCAT*-KIC, and *DrBCAT* (9 mg ml⁻¹) with L-glutamate (10 mM), referred as *DrBCAT*-L-glutamate, were mixed with an equal volume (1 μ l) of the corresponding reservoir solutions containing MgCl₂ (0.15 M), polyethylene glycol 8000 (15% [wt/vol]), and Tris-HCl buffer (0.1 M; pH 7.3), and NaCl₂ (0.2 M), polyethylene glycol 3350 (25% [wt/vol]) and bis-Tris (0.1 M; pH 6.5), respectively.

The diffraction data of the native *DrBCAT* crystal were collected to 2.5-Å resolution at 100 K at the Taiwan-contracted beamline BL12B2 of SPring-8 in Japan. The native crystals belong to space group *P*₂₁₂₁₂₁ with cell dimensions *a* = 56.37 Å, *b* = 90.70 Å, and *c* = 155.47 Å. The solvent content of crystals was estimated to be ~47.5%, with a dimer in the asymmetric unit (AU). The data sets for *DrBCAT*-KIC and *DrBCAT*-L-glutamate crystals were collected to 3.0- and 2.0-Å resolution, respectively, at 100 K at beamline BL13B1 of the National Synchrotron Radiation Research Center (NSRRC) in Hsinchu, Taiwan. Both complex crystals belong to space group *P*₂₁ but had cell dimensions *a* = 56.11 Å, *b* = 172.58 Å, and *c* = 80.08 Å for *DrBCAT*-KIC and *a* = 55.88 Å, *b* = 75.98 Å, and *c* = 79.12 Å for *DrBCAT*-L-glutamate. The *DrBCAT*-KIC crystals contain a tetramer in the AU with a solvent content of 45.5%, whereas the *DrBCAT*-L-glutamate crystals contain a dimer in the AU with a solvent content of 38.1%. Details of the crystal diffraction and structural refinement data statistics are given in Table 2.

Determination and refinement of crystal structures. The crystal structure of native *DrBCAT* was solved by the molecular replacement (MR) method using a structure of the hBCATc monomer (PDB accession no. 2COJ) as the search model (23), which has 33% sequence identity with *DrBCAT*. Two molecules were located in the AU, confirming the dimer form. Crystallographic refinement was performed with CNS v1.2 (3). After rigid-body refinement, the solution was refined to an *R* factor of 51.63% at a resolution range from 30 to 2.5 Å.

Throughout the refinement, a randomly selected 10% of the data were set aside as a “free data set,” and the model was refined against the rest of the data with *F* ≥ 0 (4) (working data set). The composite omit electron density maps with coefficient $2F_o - F_c$ (see footnote *c* of Table 2) were calculated and visualized using O v7.0 (31), and the model was rebuilt and adjusted iteratively as required. After several cycles of annealing and *B*-group refinement, the *R* factor and *R*_{free} (see footnote *d* of Table 2) values decreased to 24.54 and 29.30%, respectively. The PICK-WATER subroutine from CNS was employed to define peaks in the difference maps (3 σ cutoff level) to locate water molecules. A water molecule is accepted when the identified peak correlated with a separate peak in the corresponding $F_o - F_c$ electron density map and when one (or more) hydrogen bond was identified. On the basis of these criteria, 63 water molecules were located automatically. The protein model and water molecules were then subjected to another run of positional, simulated annealing and *B*-factor refinement. For the data with resolution between 30 and 2.5 Å, the resulting model yielded a final *R* factor of 23.34% and *R*_{free} of 28.38% (Table 2).

The refinement procedures of *DrBCAT*-KIC and *DrBCAT*-L-glutamate structures were similar to those of the native *DrBCAT* structure. The structures of *DrBCAT*-KIC and *DrBCAT*-L-glutamate were solved with the molecular replacement method using the determined native *DrBCAT* monomer as the search model. The tetramer and dimer molecules were located in the asymmetric unit of *DrBCAT*-KIC and *DrBCAT*-L-glutamate crystals, respectively. The models of KIC and L-glutamate with clearly observable electron densities were built at the active site. A Ramachandran plot was generated using MolProbity to examine backbone dihedral angles of amino acids in the favored, generally allowed, and disallowed regions for all refined models (9, 46). All refinement statistics are given in Table 2.

TABLE 2 Statistics of crystal diffraction and structural refinement of *DrBCAT* and complexes with L-glutamate and KIC

Statistic	Value ^a for:		
	Native <i>DrBCAT</i> alone	<i>DrBCAT</i> in complexes with:	
		L-Glutamate	KIC
Crystal diffraction statistics			
Wavelength (Å)	1.00	1.00	1.00
Temp (K)	100	100	100
Resolution range (Å)	2.5–30	2.0–30	3.0–30
Space group	<i>P</i> ₂ ₁ ₂ ₁ ₂ ₁	<i>P</i> ₂ ₁	<i>P</i> ₂ ₁
No. of unique reflections	28,352	53,244	34,610
Completeness (outermost shell) (%)	98.6 (100)	99.4 (97.7)	97.0 (96.2)
<i>I</i> / σ (outermost shell)	34.8 (4.5)	26.78 (2.9)	11.52 (3.64)
Average redundancy	6.2	4.9	4.4
<i>R</i> _{sym} ^b (%)	3.9	6.0	14.0
Mosaicity	0.41	0.56	0.50
Unit-cell parameter (Å)			
<i>a</i>	56.37	55.88	56.11
<i>b</i>	90.70	75.98	172.58
<i>c</i>	155.47	79.12	80.08
Structural refinement statistics			
<i>R</i> ^c (%)	23.34	22.10	20.42
<i>R</i> _{free} ^d (%)	28.38	26.30	27.11
RMSD			
Bond length (Å)	0.007	0.006	0.007
Bond angle (°)	1.318	1.270	1.387
No. of:			
Amino acids	358	358	358
Molecules per AU	2	2	4
Water molecules	63	156	177
Average <i>B</i> -factor (Å ²)	43.30	25.09	26.27
Ramachandran plot (favored regions/allowed regions/outlier regions) (%)	90.3/99.2/0.7	94.3/100.0/0.0	96.7/99.7/0.2

^a The values in parentheses are the values for the outermost shell.

^b $R_{\text{sym}} = \sum_h \sum_i |I_i(h) - \langle I(h) \rangle| / \sum_h \sum_i I_i(h)$ where I_i is the *i*th measurement and $\langle I(h) \rangle$ is the mean weight of all measurements of $I(h)$. The reflection cutoff ($I/\sigma > 0$) was applied in generating the statistics.

^c $R = \sum_h |F_o - F_c| / \sum_h F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes of reflection *h*.

^d $R_{\text{free}} = \sum_h ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_h |F_{\text{obs}}|$ for 5% of the reserved reflections.

Protein structure accession numbers. The atomic coordinates and structure factors have been deposited in the Protein Data Bank with accession numbers 3UYY, 3UZO, and 3UZB for native *DrBCAT*, *DrBCAT*-L-glutamate, and *DrBCAT*-KIC, respectively.

RESULTS

Enzyme activity and substrate specificity. The purified protein with a high concentration showed a yellow color, indicating that PLP was incorporated in the active site. The activity assay showed that the recombinant native *DrBCAT* exhibits a specific activity of 180 U/mg using α -ketoisocaproate and L-glutamate as the substrates. The specific activity of *DrBCAT* is 30 to 40 times that of the HB8BCAT (4 U/mg) and eBCAT (6 U/mg) (Table 3). The K202R and R145E single mutants and the Y71A/R145A double mutant of *DrBCAT* exhibit no specific activity (Table 3), confirming that these amino acids are the key residues for the enzymatic activity.

We compared the catalytic powers of *DrBCAT*, eBCAT, and

TABLE 3 Specific activities of *Dr*BCAT, *Dr*BCAT mutants, HB8BCAT, and *e*BCAT

Protein	Sp act ($\mu\text{mol/min mg}$)
<i>Dr</i> BCAT	
Wild type	180
Mutants	
Y71A/R145A	0
K202R	0
R145E	0
HB8BCAT	4
<i>e</i> BCAT	6

HB8BCAT with several various α -keto acid substrates, including α -ketoisocaproate (KIC) (4-methyl-2-oxopentanoate), α -keto- β -methylvalerate (KMV) (3-methyl-2-oxobutanoate), α -ketoisovalerate (KIV) (3-methyl-2-oxopentanoate), ketomethiobutyrate (KMTB), 2-ketohexanoic acid, and 2-oxovaleric acid, using L-glutamate as the common amino group donor (Table 4). Among these substrates, KIC showed the greatest relative activity as the best substrate for *Dr*BCAT, *e*BCAT, and HB8BCAT. With the relative activity of KIC set at a standard 100%, the 2-ketohexanoic acid and 2-oxovaleric acid showed a catalytic power greater than 50%. For KMV, the substrate specificities of *Dr*BCAT and HB8BCAT were less than that of *e*BCAT, but our data showed that the *Dr*BCAT, *e*BCAT and HB8BCAT could catalyze BCAA of any kind and could also react with KMTB. These results also showed that the substrate specificity of BCAT for KMTB was small. KMTB presumably does not comprise all hydrophobic groups, making the relative activity of BCAT for KMTB less than that for KIC.

Radiation effect and thermostability. Comparing the rates of survival of *D. radiodurans* with *E. coli* exposed to 2.5 kGy gamma ray, *D. radiodurans* is extremely resistant to ionizing radiation that does not influence its survival, but the survival rate of *E. coli* was decreased to about 10^{-4} by 2.5 kGy gamma ray (12). The activities of *Dr*BCAT, *e*BCAT, and HB8BCAT were measured and compared after exposure to X-rays with 718.3 rads per min for 6 h; the total dosage of X-rays for 6 h was about 2.58 kGy. When the concentrations of *Dr*BCAT, *e*BCAT, and HB8BCAT were $\sim 0.5 \text{ mg ml}^{-1}$, the activities remained ~ 59 , 45, and 49% after X-ray radiation, respectively (Table 5). The analysis shows that *Dr*BCAT

TABLE 5 Remaining activities of BCAT after X-ray exposure

Protein	Concn (mg ml^{-1})	Remaining activity (%) ^a
<i>Dr</i> BCAT	0.4978	58.79
<i>e</i> BCAT	0.4936	44.88
HB8BCAT	0.5217	48.64

^a The activity of unexposed protein is 100% for the reference.

resists radiation better than *e*BCAT and HB8BCAT. Moreover, our measurements show that the thermal stability of HB8BCAT becomes greater than those of *e*BCAT and *Dr*BCAT above 60°C, as shown in Fig. 2. We discuss the reason in the Discussion.

Overall structure of *Dr*BCAT. The crystal structure of native *Dr*BCAT was determined at a resolution of 2.5 Å (Table 2). The asymmetric unit of space group $P2_12_12_1$ contains two *Dr*BCAT molecules with the essentially identical structure upon superimposition. The first 23 amino acids at the N-terminal region were disordered and invisible in the density map. The overall structure of the *Dr*BCAT monomer comprises two domains—a small domain (Ile24 to Pro171) and a large domain (Ile186 to Val358), which are linked by an interdomain loop (Val172 to Phe185) located on the molecular surface (Fig. 3A). The fragment from Gly173 to Gly179 exhibits a dynamic character in the interdomain loop near the active site. The small and large domains are folded into an open α/β structure and a pseudobarrel structure, respectively. The small domain consists of 7 β -strands ($\beta 1$ to $\beta 7$) and 3 α -helices ($\alpha 1$ to $\alpha 3$); the large domain contains 6 β -strands ($\beta 8$ to $\beta 13$) and 3 α -helices ($\alpha 4$ to $\alpha 6$) (Fig. 3A). The cofactor PLP is located at the bottom of the active site that is formed by residues at the interface of both the small and large domains and the two loops (His70 to Gln74 and Val151 to Glu162) of the small domain from other monomers, which might explain why the enzyme functions as a dimer.

The sequence alignment reveals that *Dr*BCAT exhibits small sequence identities of 32.0, 33.0, 32.5, 29.1, 42.6, and 42.6% in comparison with hBCATm, hBCATc, *e*BCAT, HB8BCAT, MtBCAT, and MsBCAT, respectively (Fig. 4). The structure of *Dr*BCAT comprises six α -helices and 13 β -strands (6 α /13 β) in total. An inspection of other BCAT structures shows varied compositions of the secondary structures in hBCATm (8 α /20 β), hBCATc (6 α /20 β), *e*BCAT (9 α /14 β ; refer to PDB accession no. 1A3G), HB8BCAT (11 α /21 β), MtBCAT (9 α /17 β), and MsBCAT

TABLE 4 Substrate specificities of *Dr*BCAT, *e*BCAT, and HB8BCAT

α -Keto acid substrate ^a	<i>Dr</i> BCAT		<i>e</i> BCAT		HB8BCAT	
	Relative activity (%)	Sp act	Relative activity (%)	Sp act	Relative activity (%)	Sp act
KIC	100.0	154.44	100.0	5.26	100.0	8.45
KIV	62.5	96.57	36.5	1.92	41.6	3.51
KMV	34.9	53.95	61.5	3.24	28.3	2.39
KMTB	22.7	34.98	26.4	1.39	14.5	1.22
2-Ketohexanoic acid	57.1	88.20	78.4	4.12	30.7	2.60
2-Oxovaleric acid	51.0	78.84	66.9	3.52	23.5	1.99

^a L-Glutamate as an amino group donor reacted with α -keto acid. The activity of KIC was set at 100%. Abbreviations used: KIC, α -ketoisocaproate; KMV, α -keto- β -methylvalerate; KIV, α -ketoisovalerate; KMTB, ketomethiobutyrate.

FIG 2 Comparison of thermal stabilities of various BCATs. The activity of untreated BCAT enzyme was set at 100%. BCATs were incubated at each temperature for 10 min before measurement. The remaining activities were determined on measuring the temporal decrease of NADH absorbance at 340 nm at 25°C. The activity data of *Dr*BCAT (squares), *e*BCAT (circles), and HB8BCAT (triangles) are indicated.

FIG 3 Crystal structures of *DrBCAT* and molecular packing. (A) The PLP-bound monomer structure of *DrBCAT* is shown with six α -helices (turquoise) and 13 β -strands (magenta). The overall structure comprises small and large domains, connected to the interdomain loop (Val172 to Phe185; shown in black) located on the molecular surface. The cofactor PLP (dark blue stick) is located at the bottom of the active site between the two domains. (B) The *DrBCAT*-L-glutamate dimer (space group $P2_1$) is shown from a direction nearly perpendicular to the 2-fold axis. The PLP (dark blue stick) and L-glutamate (red stick) are located at the bottom of the active site.

(7 α /19 β). Superimposed structures of *DrBCAT* and other BCAT enzymes reveal structural deviations with large root mean square deviation (RMSD) values (in angstroms) for hBCATm (7.21), hBCATc (20.20), eBCAT (17.26), HB8BCAT (17.40), MtBCAT (7.21), and MsBCAT (7.18) for all main-chain atoms.

Active site, essential residues, and PLP binding. The sequence analysis and alignment of *DrBCAT* with other BCAT enzymes from various species indicated that the conserved residue Lys202 in *DrBCAT* is the catalytic residue (Fig. 4). An inspection of the *DrBCAT* structure reveals that this residue, Lys202, on the loop before the α 4 helix, is located in the active site. The electron density analysis clearly reveals that the PLP cofactor is located at the bottom of the active site between two domains. This positively charged active site pocket with a width of 9.3 Å and depth of 9.8 Å is formed and surrounded by residues Phe76, Glu77, Gly78, Lys80, Arg100, Tyr143, Arg145, Lys202, Tyr207, Glu238, Ala241, Leu263, Thr304, Ala305, Tyr71*, and Val157* (an asterisk indicates that the residue is from another monomer), and residues Val172 and Tyr175 at the interdomain to cover partially the active site (Fig. 5A).

The PLP cofactor is located between the main chain of Ala241 from the *si*-face of PLP and the side chain of Leu263 from the *re*-face of PLP, which is composed of the sandwich form, in the

active site pocket. The PLP is bound to the active site pocket mainly with hydrogen bond and salt bridge interactions mediated by residues Arg100, Tyr207, Glu238, Ser265, Ile266, Thr267, and Thr304 in *DrBCAT* (Fig. 6A) (43, 59). These residues, except Glu238, Ser265, Ile266, and Thr267, are conserved for PLP binding in the active site based on the sequence analysis and alignment of *DrBCAT* with hBCATm, hBCATc, eBCAT, HB8BCAT, MtBCAT, and MsBCAT (Fig. 4). The crystal structures of native eBCAT and hBCAT with only PLP were previously determined, allowing us to compare the interaction differences between PLP and residues of *DrBCAT*, eBCAT (42), and hBCATm (59). In the PLP binding site of the native *DrBCAT*, the observed water molecules form no hydrogen bond with PLP at a resolution of 2.5 Å, which is different from eBCAT and hBCATm with some water-mediated hydrogen bonding at a similar resolution. The detailed interactions between residues and the PLP molecule are shown in Fig. 6A. The hydrogen bonding between the phosphate OP3 of PLP and the hydroxy group of Ser265 in *DrBCAT* was not observed in both eBCAT and hBCATm, and OP3 formed no hydrogen bond with the hydroxy group of Thr267 in the hBCATm. OP4 has a hydrogen bond with the guanidino group of Arg100, differing from eBCAT and hBCATm.

The NH_2 of the catalytic residue Lys202 is not covalently linked to C4A (C4') of the PLP, which differs markedly from the cases of eBCAT and hBCATm that exhibit covalent bonds of length of ~ 1.37 Å between Lys and the PLP-C4A atom (59). Lys202 lies on the *re*-face of the PLP with the interacting distance in a range from 3.06 to 4.22 Å for all crystal structures of *DrBCAT* in this work. With C5-C5A (C5') in the PLP as the axis of rotation, the PLP in *DrBCAT* is rotated 48° and 19° relative to PLP molecules of eBCAT and hBCATm, respectively, which primarily makes the interaction distance greater than the covalent bond between C4A of PLP and NH_2 of Lys202.

Complex structures of the *DrBCAT*-substrate and substrate binding. To observe the substrate molecules bound in the active site, we cocrystallized *DrBCAT* with various substrates. The structures of *DrBCAT* complexes with L-glutamate and KIC were solved at a resolution of 2.0 and 3.0 Å, respectively (Table 2). The *DrBCAT*-L-glutamate complex exists as a dimer in an asymmetric unit of a crystal form $P2_1$ (Fig. 3B), whereas the *DrBCAT*-KIC complex exhibits a tetramer in the same space group but with a longer *b* axis (double length). Both *DrBCAT* complexes clearly reveal electron densities of the L-glutamate and KIC substrates bound near the cofactor PLP inside the active site pocket (Fig. 5B and C). The enzyme-substrate complexes reveal several essential residues for the substrate binding, of which some are conserved in all BCAT enzymes from various species (Fig. 4). A structural comparison revealed that no significant conformational variations are observed between the backbone structures of *DrBCAT* with and without substrates, except for the dynamic loop (residues 173 to 179) that exhibits the close and open forms, which is described in the next section.

In *DrBCAT*-L-glutamate, Tyr71*, Ile155*, Val157*, Arg145, Tyr175, Ala241, Thr304, Ala305, and Ala306 form the substrate-binding site and share hydrogen bonds with L-glutamate. L-Glutamate contains four carboxylate oxygen atoms, of which two are α -carboxylate oxygen atoms and two are γ -carboxylate oxygen atoms, which interact with the surrounding residues mainly through hydrogen bonds. The detailed interactions between resi-

FIG 4 Structure-based sequence alignment and comparison of BCATs from various species. From top to bottom, the BCATs were from *Deinococcus radiodurans* (DrBCAT), human cytosolic (hBCATc), human mitochondrial (hBCATm), *Mycobacterium smegmatis* (MsBCAT), *Mycobacterium tuberculosis* (MtBCAT), *Escherichia coli* (eBCAT), and *Thermus thermophilus* HB8 (HB8BCAT). The secondary structures are defined according to the DrBCAT structure.

dues, PLP, and the L-glutamate molecule are shown in Fig. 6B (also data not shown).

In DrBCAT-KIC, Gly78, Tyr143, and Lys202 form hydrogen bonds with KIC at the substrate-binding site. The detailed network of hydrogen bonds between residues, PLP, and the KIC molecules is shown in Fig. 6C (also data not shown). The position and orientation of the KIC substrate in DrBCAT and hBCATm-C315A-C318A vary because different residues form hydrogen bonds with KIC in DrBCAT and hBCATm-C315A/C318A (58).

At the PLP binding site of the DrBCAT complexes, the PLP bonds are almost the same in the DrBCAT-KIC and DrBCAT-L-glutamate with exception of only one residue, Tyr207 (Fig. 6B and C). O3 of PLP forms hydrogen bonds with the NH₂ of Lys202 and the hydroxyl group of Tyr207 in DrBCAT-L-glutamate. The hydrogen bond between O3 of PLP and OH of Tyr207 is absent from DrBCAT-KIC. The interactions of other residues and PLP in DrBCAT complex are similar to those in native DrBCAT (data not shown).

Interdomain loop (residues Val172 to Phe185) near the active site. A comparison of native and complex structures (DrBCAT-L-glutamate and DrBCAT-KIC) shows that the overall structures are similar except for one dynamic loop (Gly173 to

Gly179), structurally located near the active site, in the interdomain region (Val172 to Phe185) after the strand $\beta 7$ to connect the small and large domains (Fig. 3A). An inspection of the distributions of the temperature factor in the structures of the native and complex forms shows high B-factors with average values of 67.47, 46.6, and 40.26 Å² at this region for native DrBCAT, DrBCAT-L-glutamate, and DrBCAT-KIC, respectively, indicating that the loop is structurally dynamic. This loop is originally orientated away from the PLP by ~14.1 Å (a distance measured from main-chain C α of Tyr175 to C4A of the PLP cofactor) at the active site in the native DrBCAT (an open form for the precatalyzing stage); upon binding to the L-glutamate or KIC substrate, the loop shifts toward the PLP (a close form for the catalyzing stage) (Fig. 7A). The dynamic loop is notable because the substrate binding induced its maximum conformational shift ~5.8 Å from the substrate-free state to the substrate-bound state (Fig. 7A). Residues Tyr175 and Phe176, located on this dynamic loop to form a substrate channel from the surface to the active site, exhibit an edge-to-face interaction in the native DrBCAT, DrBCAT-KIC, and DrBCAT-L-glutamate.

A comparison of the active sites and interdomain loops of DrBCAT, eBCAT, and HB8BCAT shows some varied structural

FIG 5 Active site of native *DrBCAT*. (A) Stereo view of the active site residues. The PLP (dark blue) is located at the bottom of the active site pocket surrounded by the residues from varied orientations—at the bottom (green), near the structural surface (red), at the interdomain loop (turquoise), and at the positions behind and above the aldehyde group of PLP (yellow). (B) Cofactors and substrates in the active sites of the *DrBCAT*–L-glutamate complex. (C) Cofactors and substrates in the active sites of *DrBCAT*–L-KIC. The omit electron density maps (contoured $\geq 1.0 \sigma$) with the coefficient $2F_o - F_c$ were calculated for *DrBCAT*–L-glutamate at a resolution of 2.0 Å and for *DrBCAT*–KIC at a resolution of 3.0 Å, respectively.

features (Fig. 7B). In the native *eBCAT*, the interdomain loop (Trp126 to Gln137), which was not definable in the electron density map, is highly flexible. When substrates bound to the active site, the flexible loop became visibly located near the active site in the *eBCAT*–4-MeVA (4-MeVA stands for 4-methylvalerate) and *eBCAT*–L-glutamate (22, 43). A similar dynamic feature of the interdomain loop (Trp126 to Val135) in HB8BCAT was also observed. In the *DrBCAT*, however, the interdomain region containing the dynamic loop in our three structures—native *DrBCAT*, *DrBCAT*–KIC, and *DrBCAT*–L-glutamate are all definable and visible with varied conformations in the electron density maps (Fig. 7A).

DISCUSSION

Oligomerization of BCAT and structural implication for thermostability. As mentioned previously, native *DrBCAT* and *DrBCAT*–L-glutamate complex exhibit a dimer form, whereas *DrBCAT*–KIC molecules pack into a tetramer form that is composed of two basic dimers in the crystals (Fig. 3B). In contrast with *DrBCAT*, hBCATc, hBCATm, MsBCAT, and MtBCAT that form dimers, *eBCAT* (42) and HB8BCAT (PDB accession no. 1WRV), which equip the shorter N terminus with ~ 40 fewer residues, have been shown to be hexamers that comprise three dimers with crystallographic 3-fold axes perpendicular to 2-fold axes. Previous work showed that *eBCAT* is a hexamer in aqueous solution (35),

FIG 6 Schematic diagram of interaction networks with hydrogen bonds and salt bridges among the PLP, substrates, and residues. (A) Interactions for the native *DrBCAT* monomer. (B) Interactions for the *DrBCAT*–L-glutamate complex. (C) Interactions for the *DrBCAT*–KIC complex. The PLP as a cofactor was located at the bottom of the active site pocket with its *re*-face toward the *DrBCAT*. The interactions of hydrogen bonds (broken lines) and salt bridges (lines made of a long dash, a dot, and a long dash) are shown.

whereas we demonstrated that *DrBCAT* and HB8BCAT are a dimer and a hexamer, respectively, in aqueous solution using a gel filtration chromatography. A comparison of the dimeric, hexameric, and tetrameric molecular packing shows that all crystal forms of BCAT have a similar dimer unit. The basic dimer molecules are greatly stabilized by the network of hydrogen bonds between monomer A and monomer B in native *DrBCAT* and *DrBCAT*–L-glutamate with 27 and 31 hydrogen bonds, respectively, of which 23 and 24, respectively, are within the range of van der Waals forces (< 3.5 Å) (data not shown).

In *DrBCAT*–KIC, the *DrBCAT* tetramer is formed by mono-

FIG 7 Molecular surface of the active site and interdomain loop. (A) Structural comparison of the active sites of native *DrBCAT* (blue), *DrBCAT*-L-glutamate (green), and *DrBCAT*-KIC (red). The active site exhibits an open form in native *DrBCAT*, whereas it becomes a closed form once the L-glutamate substrate or KIC binds to the active site, because of the dynamics and flexibility of the interdomain loop. The composite omit electron density maps with coefficient $2F_o - F_c$ of the interdomain loops are shown for native *DrBCAT*, *DrBCAT*-L-glutamate, and *DrBCAT*-KIC at the bottom of panel A. (B) Structural comparison of native *DrBCAT*-PLP, *eBCAT*-PLP, and *HB8BCAT*-PMP. Superimposing the structures of the native *DrBCAT* (blue), *eBCAT* (salmon), and *HB8BCAT* (orange) reveals that the interdomain loop is highly flexible and disordered without electron densities in *eBCAT* and *HB8BCAT*, whereas the loop is clearly visible with large *B*-factors in *DrBCAT*. The N-terminal loop in *DrBCAT* is longer than those in *eBCAT* and *HB8BCAT*. Residues Tyr175 and Phe176 exhibit an edge-to-face interaction in the interdomain loop in *DrBCAT*. The black circles indicate the disordered ends of the interdomain loops of *eBCAT* and *HB8BCAT*.

mers A, B, C, and D. There are 26 hydrogen bonds (19 van der Waals forces) at the interface between monomers A and B and 24 hydrogen bonds (18 van der Waals forces) between monomers C and D. There are three hydrogen bonds (three van der Waals forces) between monomers A and C and five hydrogen bonds (three van der Waals forces) between monomers B and D. The oligomerization might explain why the thermal stabilities in *eBCAT* and *HB8BCAT* are generally greater than that in *DrBCAT* (Fig. 2), because the hexamers (*eBCAT* and *HB8BCAT*) exhibit more intermolecular interactions than a dimer (native *DrBCAT*).

FIG 8 Superposition of the PLP, substrate, and lysine residues from the *DrBCAT*, *hBCATm*, *hBCATc*, *HB8BCAT*, and *eBCAT*. (A) Stereo view of a conformational comparison of the PLP, substrate, and Lys (PLP-substrate-Lys) in native *DrBCAT* (blue-gray), *DrBCAT*-L-glutamate (green), and *DrBCAT*-KIC (red) complexes. (B) Structural comparison of PLP-substrate-Lys of *DrBCAT*-L-glutamate (green), *eBCAT*-L-glutamate (salmon), and *HB8BCAT*-L-glutamate (orange, on the bottom). (C) Structural comparison of PLP-substrate-Lys of *DrBCAT*-KIC (red), *hBCATm*-C315A-C318A-KIC (magenta), *eBCAT*-4-MeVA (orange), oxidized *hBCATc*-4-MeVA (blue), and *HB8BCAT*-4-MeVA (yellow). The Lys202 is noncovalently linked to the PLP with distances of 3.06, 3.16, and 4.22 Å between C4A of PLP and NZ of Lys202 for native *DrBCAT*, *DrBCAT*-L-glutamate, and *DrBCAT*-KIC, respectively.

Our data show that the thermal stability of *HB8BCAT* is greater than that of *eBCAT* and *DrBCAT* above 60°C despite the fact that both *HB8BCAT* and *eBCAT* are hexamers. A structural comparison of *DrBCAT*, *eBCAT*, and *HB8BCAT* monomers revealed that *HB8BCAT* contains 11 α -helices, while *DrBCAT* has 6 α -helices and *eBCAT* has 9 α -helices (PDB accession no. 1A3G). A careful inspection of structures among *HB8BCAT*, *eBCAT*, and *DrBCAT* shows that *HB8BCAT* exhibits two additional small α -helices—an α -helix from Leu112 to Asn114 forms five hydrogen bonds with the neighbor loop (Gly103 to Pro111) and residue Asn115 and an α -helix from Glu299 to Trp301 forms six hydrogen bonds with the neighbor loop (Pro296 to Tyr298) and the β -strand (Leu302 to Tyr304). In contrast, there are only the loop conformations at these two corresponding α -helix regions in *HB8BCAT* forming fewer hydrogen bonds in *DrBCAT* (Thr159 to Pro161 and Pro351 to Gly353) and *eBCAT*. More hydrogen bonds between secondary structures could give *HB8BCAT* the greatest temperature of thermal denaturation and the greatest stability among the three enzymes (29, 54).

PLP binding and substrate binding of BCATs. Comparison of the active site structures of the native *DrBCAT*, *DrBCAT*-L-glutamate, and *DrBCAT*-KIC complex indicates that all bonds between Lys202 and C4A of PLP with distances of 3.06, 3.16, and 4.22 Å, respectively, are not covalently linked (Fig. 8A, B, and C). The noncovalent linkage between the Lys residue and PLP was previously proposed to be an effect of radiation damage (15, 55), but we cannot exclude the possibility of a noncovalent linkage as the nat-

ural character and state for *DrBCAT* from the radiation-resistant *D. radiodurans*.

The structures of the BCAT-PLP complex with L-glutamate were previously determined in *E. coli* (22) and *T. thermophilus* (PDB accession no. 2EJ2). In our *DrBCAT*-L-glutamate, the guanidine group of L-glutamate does not connect with C4A (C4') of PLP with a distance of ~ 3.78 Å, which is larger than those of *eBCAT* (1.53 Å) and *HB8BCAT* (1.51 Å), as shown in Fig. 8B. Because the PLP-L-glutamate has the ketimine form in the transamination reaction in *eBCAT* and *HB8BCAT*, the guanidine group of the L-glutamate might connect with C4A (C4') of PLP (Fig. 8B), but *DrBCAT*-L-glutamate is presumably in a Michaelis complex form that is C4A (C4') of PLP without connecting with the guanidine group of L-glutamate.

The complex structures of *hBCATm*-C315A/C318A-KIC and *hBCATm*-PMP-KIV were solved previously (58). The structures of *eBCAT* (43), oxidized *hBCATc* (23), and *HB8BCAT* complex with substrate analogue 4-MeVA (PDB accession no. 2EIY; unpublished work) as the Michaelis complex form were also determined. The *DrBCAT*-KIC complex also has a Michaelis complex form in the transamination reaction. The results of comparison are shown in Fig. 8C. A Schiff base between Lys and the PLP of other BCAT enzymes (*eBCAT*-4-MeVA, *hBCATm*-C315A/C318A-KIC, oxidized *hBCATc*-4-MeVA, and *HB8BCAT*-4-MeVA) are generally observed to have a Michaelis complex form.

Putative role of the interdomain loop of BCATs. The structural and sequence comparisons of *DrBCAT*, *eBCAT*, and *HB8BCAT* show that the N-terminal segment (amino acids [aa] 1 to 39) before $\beta 1$ in *DrBCAT* is longer than the segments in *eBCAT* and *HB8BCAT* (Fig. 4 and 7B). The oxygen atom of Gly30 on the N-terminal loop forms a hydrogen bond (distance of 2.8 Å) with the main-chain NH group of Gly173 in the interdomain loop in native *DrBCAT*, *DrBCAT*-KIC, and *DrBCAT*-L-glutamate. The longer N-terminal loop might limit the flexibility of the dynamic interdomain loop in the native state of *DrBCAT* because of the spatial obstacle (Fig. 7B). Similar phenomena were observed in *hBCATm*, *hBCATm*, and *MsBCAT*.

The native *DrBCAT* containing a more stable interdomain region with the open conformation exhibits a specific activity greater than those of *HB8BCAT* and *eBCAT* (Table 3). A similar character of a definable loop was observed in native *hBCATm*, which also has a large specific activity (14). In contrast, the hydroxyl group of Tyr175 in the interdomain loop interacts with the α -carboxylate oxygen atom of L-glutamate in *DrBCAT*-L-glutamate. As a result of the above reasons, we speculate that the existence of the interdomain loop might affect the rate of reaction or electron transfer would occur between Tyr175 and the substrate. Several substrates, including KIC, KIV, KMV, KMTB, 2-ketohexanoic acid, and 2-oxovaleric acid, have been proven to be substrates of *DrBCAT*, despite varied substrate specificities as previously discussed. The flexibility of the interdomain loop could further explain variations of substrates for the BCAT enzyme, as the space for the substrate entrance could be adjustable.

Protective effect of cysteine and radiation resistance. Under concentrations of ~ 0.5 mg ml⁻¹, the activities remain ~ 59 , 44, and 48% in *DrBCAT*, *eBCAT*, and *HB8BCAT*, respectively, after X-ray radiation (Table 5), implying that *DrBCAT* is more effectively protective against X-rays. The radiolysis of water with ionizing radiation produces electrons, free radicals (H $\cdot$ radicals and

$\cdot$ OH radicals) and hydrogen peroxide H₂O₂ via free radical mechanisms (50). When proteins are irradiated in aqueous solution, the reactive oxygen species, namely, $\cdot$ OH free radicals, could attack specific amino acids or the groups of amino acids, such as histidine, tyrosine, tryptophan, cysteine, and methionine, in the active site, leading to protein inactivation (19, 41, 48, 51). The residues could interact with water-derived radicals because of the readily oxidizable functional groups, including indole, phenol, aromatic, and thiol groups.

Three easily oxidizable amino acids (Tyr175, Tyr207, and Tyr71*) exist in the active site of *DrBCAT*, whereas *eBCAT* and *HB8BCAT* contain four oxidizable residues in the active site, including the three conserved Tyr residues and one additional Trp residue that is structurally related to Val172 in *DrBCAT*. The hydroxyl group of the conserved Tyr207 and O3 of PLP form a hydrogen bond in *DrBCAT*, *eBCAT*, and *HB8BCAT*. When a readily oxidizable amino acid, such as Tyr, is irradiated, free radical $\cdot$ OH might break noncovalent bonds, such as hydrogen bonds, and also damage these residues, inducing conformational modification of the secondary structure in aqueous solution (1, 48), decreasing the enzymatic activity. The additional Trp residue might make the active site more readily oxidized in *eBCAT* and *HB8BCAT*, yielding activities less than that of *DrBCAT*.

Cysteine serves as a powerful destroyer of free radicals or a scavenger of free radicals with a thiol-containing side chain that can protect against X-radiation-induced lethality base on the free radical metabolites in cysteine oxidation (7, 13, 21, 44, 47). After the free-radical scavengers (Cys) interact with free radicals, fewer free radicals remain in aqueous solution. As the probability of free radicals interacting with the readily oxidizable residues decreases, more Cys residues could give greater protection of BCAT activity.

The sequence inspection shows that *DrBCAT* contains six Cys residues in *DrBCAT*, including Cys75, Cys84, Cys105, Cys122, Cys169, and Cys302, whereas *eBCAT* has only three Cys residues and *HB8BCAT* has just one. We thus superimposed the BCAT enzymes from various species to reveal the positions and distributions of cysteines (Fig. 9). Among the six Cys residues, Cys75 and Cys302 located near the active site could protect the Tyr residues from oxidization in the active site of *DrBCAT*, whereas Cys84, Cys105, and Cys122 located on the surface of *DrBCAT* could reduce the free radicals on the molecular surface. Compared with other BCAT enzymes, *eBCAT* has two Cys residues on its surface and *HB8BCAT* has none. Because of the different numbers and positions of Cys residues, the activity of *DrBCAT* is greater than those of *eBCAT* and *HB8BCAT* after exposure to X-rays. For further comparison, *hBCATc* and *hBCATm* have five and three Cys residues located on their surfaces, respectively, and two Cys residues are found near the active sites in both enzymes (Fig. 9). We presume that *hBCATc* and *hBCATm* could also protect the enzyme with activities comparable to that of *DrBCAT* after irradiation.

The reduction of Cys residues is irreversible only in *DrBCAT*. Peroxiredoxins (Prxs) are the antioxidant enzyme, of which the reduction-oxidation reactions of Cys residues are reversible (20, 57). The cysteine (RSH) in BCAT is oxidized by H₂O₂ to a sulfenic acid (RSOH) and sulfonic acid (RSO₂H), but RSOH could be reduced to RSH with NADH-dependent enzymes (thioredoxin, AhpF, trypanoxin, or AhpD) (5, 45), and RSO₂H could reverse to RSOH with an ATP-dependent enzyme, sulfiredoxin (Srx) (2,

FIG 9 Comparison of structures and distributions of cysteine in DrBCAT, eBCAT, HB8BCAT, hBCATc, and hBCATm. The main-chain structures (thin lines), cysteine residues (sticks) and the cofactor PLP (ball-and-stick) of DrBCAT (green), eBCAT (red), HB8BCAT (purple), hBCATc (blue), and hBCATm (gray) are shown. The cysteine residues near the surface (broken-line arrow) and the active site (solid-line arrow) are indicated. Cys75 and Cys302 located near the active site might protect the Tyr residues from oxidation in the active site of DrBCAT, whereas Cys84, Cys105, and Cys122 located on the surface of DrBCAT could reduce the free radicals on the surface.

32), which might complete the catalytic cycle (redox reaction) in the BCAT enzyme upon irradiation.

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