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John T. Pinto
New York Medical College

Boris F. Krasnikov
New York Medical College


Steven Alcutt

Melanie E. Jones

Thambi Dorai
New York Medical College

See next page for additional authors

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Authors

John T. Pinto, Boris F. Krasnikov, Steven Alcutt, Melanie E. Jones, Thambi Dorai, and Arthur J L Cooper

Kynurenine Aminotransferase III and Glutamine Transaminase L Are Identical Enzymes that have Cysteine S-Conjugate β -Lyase Activity and Can Transaminate L-Selenomethionine*

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John T. Pinto^{†1}, Boris F. Krasnikov[‡], Steven Alcutt[‡], Melanie E. Jones[‡], Thambi Dorai[§], Maria T. Villar[¶], Antonio Artigues[¶], Jianyong Li^{||}, and Arthur J. L. Cooper^{†#2}

From the Departments of [†]Biochemistry and Molecular Biology and [§]Urology, New York Medical College, Valhalla, New York 10595, the [¶]Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160, and the ^{||}Department of Biochemistry, Virginia Tech, Blacksburg, Virginia 24061

Background: KAT I and GT-Kidney (K) are identical enzymes that β -eliminate and transaminate Se-methyl-L-selenocysteine (MSC).

Results: KAT III and GT-Liver (L) are identical and metabolize L-selenomethionine (SM).

Conclusion: MSC and SM are transaminated to seleno-keto acids, recognized HDAC inhibitors, by KAT/GT enzymes.

Significance: Anticancer efficacy of MSC and SM depends in part on tissue expression of KAT/GT enzymes.

Three of the four kynurenine aminotransferases (KAT I, II, and IV) that synthesize kynurenic acid, a neuromodulator, are identical to glutamine transaminase K (GTK), α -aminoadipate aminotransferase, and mitochondrial aspartate aminotransferase, respectively. GTK/KAT I and aspartate aminotransferase/KAT IV possess cysteine S-conjugate β -lyase activity. The gene for the former enzyme, GTK/KAT I, is listed in mammalian genome data banks as *CCBL1* (cysteine conjugate β -lyase 1). Also listed, despite the fact that no β -lyase activity has been assigned to the encoded protein in the genome data bank, is a *CCBL2* (synonym *KAT III*). We show that human KAT III/*CCBL2* possesses cysteine S-conjugate β -lyase activity, as does mouse KAT II. Thus, depending on the nature of the substrate, all four KATs possess cysteine S-conjugate β -lyase activity. These present studies show that KAT III and glutamine transaminase L are identical enzymes. This report also shows that KAT I, II, and III differ in their ability to transaminate methyl-L-selenocysteine (MSC) and L-selenomethionine (SM) to β -methylselenopyruvate (MSP) and α -ketomethylselenobutyrate, respectively. Previous studies have identified these seleno- α -keto acids as potent histone deacetylase inhibitors. Methylselenol (CH_3SeH), also purported to have chemopreventive properties, is the γ -elimination product of SM and the β -elimination product of MSC catalyzed by cystathionine γ -lyase (γ -cystathionase). KAT I, II, and III, in part, can catalyze β -elimination reactions with MSC generating CH_3SeH . Thus, the anticancer efficacy of MSC and SM will depend, in part, on the endogenous expression of various KAT enzymes and cysta-

thionine γ -lyase present in target tissue coupled with the ability of cells to synthesize *in situ* either CH_3SeH and/or seleno-keto acid metabolites.

The glutaminase II pathway, first elucidated by Meister and co-workers (1–4), consists of a glutamine transaminase (EC 2.6.1.–, Equation 1)³ coupled to ω -amidase (ω -amidocarboxylate amidohydrolase; EC 3.5.1.3; Equation 2) (1–10). The net reaction is shown in Equation 3. Rat tissues contain at least two glutamine transaminases, namely a liver type (glutamine transaminase L (GTL))⁴ and a kidney type (glutamine transaminase K (GTK)) (6–10).⁵ GTK is identical to kynurenine aminotransferase I (KAT I) (13–15).

³ Although the Enzyme Commission now recommends the word *aminotransferase* in place of the word *transaminase*, the word *transaminase* continues to be used for GTK. Most aminotransferases utilize glutamate as a substrate. Thus, continued use of *transaminase* for enzymes that catalyze aminotransferase reactions with glutamine may provide a useful distinction between glutamine- and glutamate-utilizing aminotransferases. Because GTK is widely used as the name for the kidney type glutamine transaminase, for consistency we use the name GTL (glutamine transaminase L) here for the liver type glutamine transaminase.

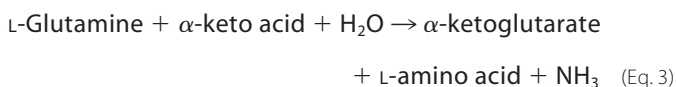
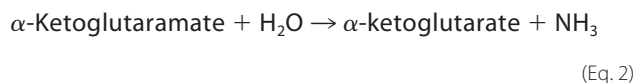
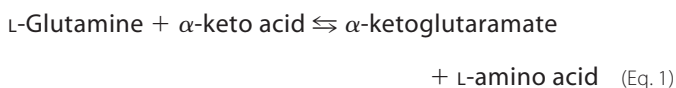
⁴ The abbreviations used are: used: GTL, glutamine transaminase L; AAT, α -aminoadipate aminotransferase; BTC, S-benzothiazolyl-L-cysteine; CCBL, cysteine S-conjugate β -lyase; DCVC, S-(1,2-dichlorovinyl)-L-cysteine; GTK, glutamine transaminase K; h, human; HDAC, histone deacetylase; KAT, kynurenine aminotransferase; KMSB, α -keto- γ -methylselenobutyrate; m, mouse; mitAspAT, mitochondrial aspartate aminotransferase; Se-methyl-L-selenocysteine; MSP, β -methylselenopyruvate; PLP, pyridoxal 5'-phosphate; SM, L-selenomethionine; TFEC, S-(1,1,2,2-tetrafluoroethyl)-L-cysteine; FDR, false discovery rate; r, rat.

⁵ The sequence of rat kidney GTK was first published by Perry *et al.* (11). Later, a slightly different sequence was reported by Abraham and Cooper (12). A BLAST analysis of the amino acid sequence in the region reported by Abraham and Cooper was found to be slightly different from that reported by Perry *et al.* and yielded no recognizable protein. Therefore, the mammalian genomes contain only one GTK gene, and the sequence reported by Abraham and Cooper most likely contained some errors in amino acid assignment.

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¹ To whom correspondence may be addressed: Dept. of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY 10595. E-mail: john_pinto@nymc.edu.

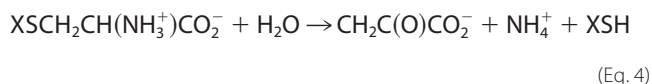
² To whom correspondence may be addressed: Dept. of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY 10595. E-mail: arthur_cooper@nymc.edu.



GTK/KAT I is one of four mammalian aminotransferases that catalyze the irreversible transamination of kynurenine to kynurenic acid, and the crystal structure of the human enzyme (hGTK/KAT I) has been elucidated (16). KAT II is identical to α -aminoadipate aminotransferase (AAT) (14, 17, 18), and KAT IV is identical to mitochondrial aspartate aminotransferase (mitAspAT) (19, 20). The KAT enzymes are of considerable interest to neurochemists because kynurenic acid is an antagonist of the *N*-methyl-D-aspartate glutamate receptor subtype, and the enzymes can be psychopharmacological targets (17, 21).

Although previous studies characterized KAT I, KAT II, and KAT IV, KAT III required further characterization. KAT I and KAT III share similar genomic structures, have high sequence homology (22, 23), and possess broad specificity toward L-amino acids and α -keto acids. Glutamine and α -keto- γ -methylbutyrate are very good amino acid and α -keto acid substrates, respectively, of mouse KAT III (mKAT III), whereas L-alanine and pyruvate are much less effective (22). mKAT III and rat liver GTL exhibit remarkably similar properties, namely, monomer size ($M_r = \sim 51,000$), subunit composition (homodimer) and substrate specificity, and optimum pH 8.5–9.0 (5). Thus, we hypothesize that GTL is identical to KAT III. In addition to comparative enzyme kinetic data, the present study provides mass spectral analysis to verify this hypothesis.

Rat kidney GTK/KAT I shows cysteine *S*-conjugate β -lyase activity depending on the substituent group in the β -position (Refs. 24 and 25; reviewed in Refs. 26 and 27). A β -elimination reaction results in the production of aminoacrylate and a sulfur-containing fragment. The aminoacrylate nonenzymatically tautomerizes to α -iminopropionate, which hydrolyzes nonenzymatically to pyruvate and ammonium. The net cysteine *S*-conjugate β -lyase reaction is shown in Equation 4.



Many electrophiles are detoxified/metabolized via the mercapturate pathway. In this pathway, the electrophile initially conjugates with glutathione, which is subsequently hydrolyzed to a cysteine *S*-conjugate, acetylated to *N*-acetyl-L-cysteine *S*-conjugate (mercapturate), and excreted in the bile and/or urine. However, if the cysteine *S*-conjugate contains a strong electron attracting group (a nucleofuge) in the β position, cysteine *S*-conjugate β -lyases readily catalyze a β -elimination reaction. β -Elimination reactions of halogenated alkene cysteine *S*-conjugates can generate sulfur-containing fragments that are highly reactive and toxic (reviewed in Ref. 26). Toxicification reactions that generate halogenated, sulfur-containing

fragments (XSH) include *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC; the cysteine *S*-conjugate of trichloroethylene) and *S*-(1,1,2,2-tetrafluoroethyl)-L-cysteine (TFEC; the cysteine *S*-conjugate of tetrafluoroethylene). By contrast, the eliminated sulfur-containing fragment of *S*-(benzothiazolyl)-L-cysteine (BTC; the cysteine conjugate of benzothiazole 2-sulfonamide) is stable and relatively nontoxic (reviewed in 26).

Following the publication by Stevens *et al.* (25), several investigators assumed that GTK is the cysteine *S*-conjugate β -lyase responsible for catalyzing β -elimination reactions with DCVC and TFEC. As a result, the gene for GTK is listed in mammalian gene data banks as *CCBL1* (cysteine *S*-conjugate β -lyase 1). In a similar fashion, a gene closely related to *CCBL1* is listed in mammalian gene data banks as *CCBL2*, despite the fact that cysteine *S*-conjugate β -lyase activity had not previously been assigned to the gene product. As noted above, this gene codes for KAT III.

We previously reported that L-methionine is a better substrate than is L-selenomethionine (SM) for recombinant human GTK (hGTK) (26, 27). Blarmino *et al.* (28), on the other hand, reported that SM is an excellent substrate of a glutamine transaminase isolated from bovine liver. We show here that SM is an excellent substrate of mGTL/KAT III. Thus, it appears that Blarmino *et al.* (28) may have isolated and characterized the L type glutamine transaminase/KAT III from bovine liver.

MSC was noted previously to be a moderately good substrate, both as an aminotransferase substrate and as a modest β -lyase substrate of rat GTK (24), a finding we verified for hGTK (27). Thus, the current study extends the distinct quantifiable differences among the KAT enzymes in their preferences for β -lyase substrates and organoselenium-containing amino acids (26).

Lastly, it was previously shown that SM is a γ -lyase substrate of cystathionine γ -lyase (29). Here we show that MSC is a β -lyase substrate of purified rat liver cystathionine γ -lyase. In both cases, the eliminated fragment is predicted to be methylselenol (CH_3SeH). Thus, the capacities of mammalian tissues to catalyze transamination and elimination reactions with seleno-amino acids are well established (30). Although methylselenol has never been isolated or measured *in situ* within tissue, it can be generated simultaneously with seleno- α -keto acid metabolites, which are measurable by electrochemical detection, as part of competing transaminase/lyase reactions that can occur at the active site of the KAT enzymes. These reactions are of considerable importance for understanding the chemopreventive efficacy of seleno-amino acids against a variety of cancers, in particular prostate and bladder. Fig. 1 illustrates the structural formulae of selected amino acid substrates of the KAT enzymes relevant to the current studies and their corresponding α -keto acid derivatives.

EXPERIMENTAL PROCEDURES

Substrate Preparations—DCVC was a gift from Dr. Robert Schwarcz (University of Maryland). TFEC was prepared by the method of Hayden and Stevens (31), and the acetate salt of BTC was prepared by the method of Cooper *et al.* (32) and was a gift from Dr. Sam Bruschi (University of Washington, Seattle, WA). MSC and SM were gifts from the Sabinsa Corporation (East Wind-

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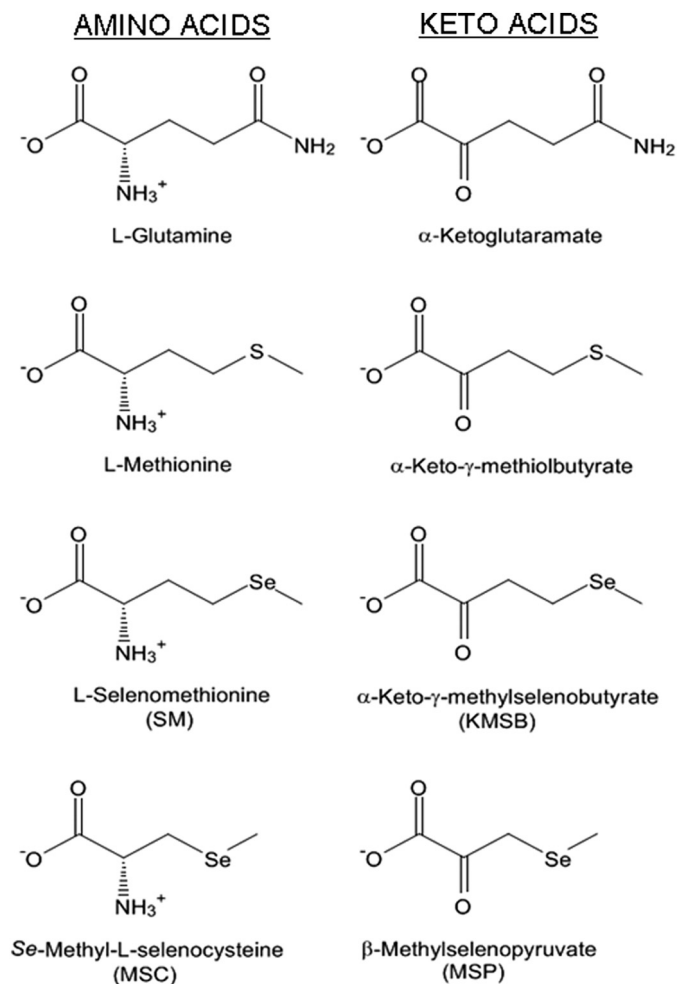


FIGURE 1. Structural formulae of selected amino acid substrates of KAT enzymes and their corresponding α -keto acid derivatives.

sor, NJ). α -Keto- γ -methiolbutyrate (systematic name: 4-(methylsulfanyl)-2-oxobutanoate; synonyms; α -keto- γ -methylthiobutyrate, 2-oxo-4-methylthiobutanoate; 4-methylthio-2-oxobutanoate) was purchased from Sigma. *S*-Allylmercapto-L-cysteine was obtained from Wakunaga of America Co. Ltd. (Mission Viejo, CA).

Enzyme Preparations—Recombinant human GTK/KAT I/CCBL1 (hGTK) was obtained by the method of Han *et al.* (33) and stored in 10 mM potassium phosphate buffer (pH 7.2) at a protein concentration of 11.2 mg/ml. Recombinant human KAT II/AAT (hKAT II) was prepared according to the method of Han *et al.* (33). The enzyme was stored in 10 mM potassium phosphate buffer (pH 7.2) at a protein concentration of 11.3 mg/ml. Recombinant mKAT III was obtained by the method of Han *et al.* (22) and stored in 10 mM potassium phosphate buffer (pH 7.2) containing 1 mM DTT at a protein concentration of 3.7 mg/ml. The enzyme is homogeneous as judged by the criterion of SDS-PAGE followed by staining with Coomassie Blue. A single band ($M_r = \sim 51,000$) was detected. All KAT enzyme solutions were stored at 4 °C. Rat liver cystathionine γ -lyase (2.4 units/mg; 1.32 mg/ml) was purified by the method of Pinto *et al.* (34). A unit of cystathionine γ -lyase activity is defined as the amount of enzyme that catalyzes the production of 1 μ mol of α -ketobutyrate per minute at 37 °C in a reaction mixture con-

taining 20 mM L-homoserine (an alternate substrate to cystathionine) and 100 mM potassium phosphate buffer (pH 7.4). Aliquots were stored frozen at -20 °C.

The purification procedure for mouse liver GTL was adapted from that of Cooper and Meister for the purification of rat liver GTL (6, 8). The following purification steps (from 20 g of liver) were followed prior to SDS-PAGE separation: 1) heat treatment, 2) acidification with acetic acid, 3) ammonium sulfate fractionation, 4) fractionation on a DE-52 anion exchange resin, and 5) fractionation on a hydroxyapatite column.

Standard Assay for GTL Transaminase Activity—L-Albizzin (a glutamine analog in which the $-\text{CH}_2-$ in the 4 position is replaced by $-\text{NH}-$) was used as the standard amino acid substrate of GTL in place of L-glutamine (6, 7, 9). Activity was measured by a modification of the method of Cooper and Meister (6–8) adapted for a 96-well plate spectrophotometer. The standard reaction mixture (0.02 ml) contained 40 mM L-albizzin, 20 mM sodium glyoxylate, 40 mM sodium pyrophosphate buffer (pH 9.0), and enzyme in a small snap top tube. After incubation for 30 min at 37 °C, the reaction was terminated by the addition of 0.18 ml of 1 M NaOH. After incubation for a further 20 min at 37 °C, the absorbance was read at 280 nm against a blank containing complete assay mixture to which enzyme was added after addition of base. Under these conditions the α -keto analog of L-albizzin cyclizes to a lactam followed by dehydration to 2-imidazolinone-4-carboxylate ($\epsilon_{280 \text{ nm}} = 10,000 \text{ M}^{-1} \text{ cm}^{-1}$) (6). A unit of GTL enzyme activity is defined as that amount of enzyme that catalyzes the formation of 1 μ mol of product (2-imidazolinone-4-carboxylate)/min at 37 °C.

Measurement of Lyase Activities— β -Lyase activity of mGTL/KAT III and KAT II toward cysteine *S*-conjugates and β -chloro-D,L-alanine was measured by a modification of the procedure of Cooper and Pinto (35). The reaction mixture (0.02 ml) contained 5 mM L-cysteine *S*-conjugate, 0.1 mM α -keto- γ -methiolbutyrate (or 0.1 mM α -ketoglutarate in the case of AAT/KAT II), 100 mM potassium phosphate buffer (pH 7.4), and enzyme. After incubation at 37 °C in a small snap top tube, the reaction was terminated by addition of 0.01 ml of 5 mM 2,4-dinitrophenylhydrazine in 2 M HCl. After additional incubation of the mixture for 10 min at room temperature, 0.17 ml of 1 M NaOH was added, and the increase in absorbance at 430 nm was read within 2 min against an appropriate blank. The blank was identical to the assay mixture except that β -lyase substrate was omitted. The molar extinction coefficient of pyruvate 2,4-dinitrophenylhydrazone under these conditions is $\sim 16,000 \text{ M}^{-1} \text{ cm}^{-1}$. A small amount of α -keto acid is included in the reaction mixture because competition of the β -lyase reaction with a half transamination reaction with PLP co-factor has the potential to generate enzyme in the pyridoxamine 5'-phosphate form, which cannot catalyze a β -lyase reaction. A half transamination reaction of α -keto with pyridoxamine 5'-phosphate will regenerate PLP, allowing the enzyme to continue to support a β -lyase reaction (24–26). An identical procedure was used to determine β -lyase activity toward β -chloro-D,L-alanine, except that it was not necessary to include an α -keto acid in the reaction mixture.

In some experiments, *S*-allylmercapto-*L*-cysteine and MSC were investigated as β -lyase substrates of cystathionine γ -lyase. The pyruvate product was measured as the 2,4-dinitrophenylhydrazone as indicated above. In other cases, *L*-homoserine, *L*-cystathionine, and SM were investigated as γ -lyase substrates of cystathionine- γ -lyase. The α -ketobutyrate product was also measured as the 2,4-dinitrophenylhydrazone (35).

All spectrophotometric measurements were carried out with a SpectraMax 96-well plate spectrophotometer (Molecular Devices, Sunnyvale, CA). All protein measurements were determined using the Bradford protein assay (Bio-Rad) or the bicinchoninic acid protein assay kit provided by Pierce using bovine serum albumin as a standard.

HPLC Quantitation of Sulfur- and Selenium-containing Compounds in Transaminase Assays—Transamination between the selenium-containing amino acids (MSC, SM) and α -keto- γ -methylolbutyrate was measured by HPLC with CoulArray (electrochemical) detection (30, 34–36). The advantage of this technique is that redox-active compounds can be quantitated without prior derivatization. Moreover, analytes can be characterized, not only by elution time but also by the voltage required for maximal oxidation. When α -keto- γ -methylolbutyrate is used as the α -keto acid co-substrate, disappearance of the selenium-containing amino acids correlates with appearance of *L*-methionine and with concomitant appearance of the selenium-containing α -keto acid. The reaction mixture (0.25 ml) contained 1 mM MSC (or 1 mM SM), 2 mM α -keto- γ -methylolbutyrate, and 50 mM potassium phosphate buffer, pH 7.4, and enzyme. The reaction mixture was incubated at 37 °C, and at 15-min intervals, 0.05 ml was withdrawn, and the reaction was quenched by addition of 0.015 ml of 25% (w/v) metaphosphoric acid. The samples were placed on ice for 15 min and centrifuged for 5 min at $21,100 \times g$ in an Eppendorf tube. The supernatant fraction was analyzed by HPLC. The seleno- α -keto acids generated via transamination of MSC and SM are β -methylselenopyruvate (MSP) and α -keto- γ -methylselenobutyrate (KMSB), respectively. Standard solutions of these seleno- α -keto acids were generated *in situ* from the corresponding *L*-amino acids with *Crotalus adamanteus* *L*-amino acid oxidase (30).

The HPLC system consisted of a liquid chromatograph equipped with an eight-channel CoulArray detector (ESA, Inc., Chelmsford, MA) (35, 36). Sample aliquots are injected directly onto a Bio-Sil ODS-5S, 5- μ m particle size, 4.0×250 mm, C18 column (Bio-Rad Life Science Research Group) and eluted with a mobile phase consisting of 50 mM NaH_2PO_4 , 50 μ M octane sulfonic acid, and 3% (v/v) acetonitrile (pH 2.62) at a flow rate of 1 ml/min. All buffers following preparation are routinely degassed and filtered through a 0.2- μ m Millipore nylon filter, and the pH was adjusted, if necessary. PEEKTM (polyetheretherketone) tubing was used throughout the HPLC system, and a 0.2- μ m PEEKTM filter was placed pre- and post-column to protect both column and flow cells, respectively, from any particulate matter. A Rheodyne injection valve with a 5- μ l sample loop was used to manually introduce samples. The eight channels of the CoulArray detector were set at 350, 450, 550, 700, 750, 800, 850, and 900 mV, respectively. Retention times and detection potential ranges are: MSC (4.11 min, 700–850 mV); MSP (4.56 min, 700–850 mV); *L*-methionine (5.32 min, 750–850 mV), α -keto-

γ -methylolbutyrate (5.93 min, 700–850 mV); SM (6.58 min, 550–750 mV); and KMSB (7.65 min, 550–750 mV). Peak areas under the concentration curves of chromatograms for each reaction mixture were compared against standards of the amino and α -keto acids from 2 to 200 nmol/ml for each compound mentioned above. Except where noted, all enzyme activity measurements were carried out at least in triplicate and are reported as the means \pm S.E.

SDS-PAGE—Samples were mixed with complete $2 \times$ SDS-denaturing electrophoresis sample buffer (Bio-Rad) and boiled for 6 min. Solubilized proteins were processed to remove interfering substances with a Compat-Able protein assay preparation reagent (Thermo-Scientific). Equal amounts (20 μ g) of protein were loaded onto each lane of a commercially available pre-cast gel (Criterion TGX, 4–15% gradient; Bio-Rad) with Kaleidoscope molecular weight standards (Bio-Rad). The gel was stained with 0.25% Coomassie Blue R-250 in 50% trichloroacetic acid, followed by destaining with 25% methanol, 7% acetic acid. The stained band corresponding to the position of GTL based on its known M_r was excised for MS analysis.

Mass Spectrometry—The excised band was reduced, alkylated, and digested with trypsin. The resulting peptides were extracted and analyzed by reversed phase chromatography using an Eksigent nano HPLC, equipped with a Famos autosampler, connected to a linear trap quadrupole-Fourier transform mass spectrometer. The peptides were loaded onto a peptide trap column (3 cm \times 0.1 μ m) packed in-house with C18 reversed phase material (Magic C18, 5 Å, 200 μ m), the peptides were desalted for 10 min using 0.1% formic acid at a flow rate of 3 μ l/min. Following desalting the peptide trap column was connected to an analytical reversed phase column (12 cm \times 75 μ m) packed in house with the same material. The peptides were eluted by means of a 0.1% formic acid (solvent A) and 0.1% formic acid in acetonitrile (solvent B) mixture, using the following gradient: 5–15% B in B + A for 5 min, 15–50% B in B + A over the next 175 min, followed by an 80% B wash for 10 min at a flow rate of 250 nl/min.

The mass spectrometer was operated in automatic mode using Xcalibur software (version 3.0) programmed to perform one parent mass scan on the Fourier transform spectrometer at 50,000 resolution (for an ion with an m/z of 400), followed by six tandem MS scans on the most intense ions, using a repeat of two in 0.5 min and an exclusion list of 1.5 min. Tandem mass spectra were extracted, and the charge state was deconvoluted by Xcalibur software (version 3.0).

Database Searching and Protein Identification—All MS/MS spectra were analyzed using SequestHT (Thermo Fisher Scientific, San Jose, CA; version 1.4.0.288) to search a mouse protein database derived from the National Center for Biotechnology Information repository (2014 04 11; 377,628 entries) assuming the digestion enzyme trypsin, using a fragment ion mass tolerance of 0.60 Da and a parent ion tolerance of 20 ppm. Iodoacetamide was used to alkylate cysteine that results in the covalent addition of a carbamidomethyl group (57.07 Da). Carbamidomethylation of cysteine was specified as a fixed modification. Deamidation of asparagine and glutamine, cyclization of glutamine and glutamate to pyroglutamate (5-oxoproline), oxidation of methionine, and acetylation of the N terminus

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TABLE 1

Co-purification from mouse liver of L-albizzin/glyoxylate transaminase activity with SM/ α -keto- γ -methiolbutyrate transaminase activity, but not with MSC/ α -keto- γ -methiolbutyrate transaminase activity

The purification steps (from 20 g of mouse liver) were as follows: 1) heat treatment, 2) acidification with acetic acid, 3) ammonium sulfate fractionation, 4) fractionation on a DE-52 anion exchange resin, and 5) fractionation on a hydroxyapatite column. All enzyme activity and protein measurements were carried out in duplicate or triplicate. For reaction mixtures containing SM/ α -keto- γ -methiolbutyrate, HPLC separation of both methionine and KMSB equimolar concentrations were determined within time points from 10 to 60 min. In a similar fashion, for reaction mixtures containing MSC/ α -keto- γ -methiolbutyrate, HPLC separation of both methionine and MSP revealed equimolar concentrations within time points from 10 to 60 min. The percentage yield at step 1 was arbitrarily set at 100%.

Step	L-Albizzin/glyoxylate transaminase						SM/ α -keto- γ -methiolbutyrate transaminase			MSC/ α -keto- γ -methiolbutyrate transaminase	
	Volume	Protein	Activity (A)	Yield	Specific activity	Fold purification	Activity (B)	Specific activity	B/A	Activity (C)	C/A
	ml	mg	milliunit	%	milliunit/mg		milliunit	milliunit/mg		milliunit	
1	100	1060	5133	100	4.84	1	4683	4.42	0.91	2532	0.49
2	73	542	4185	82	7.72	1.58	2976	5.49	0.71	1079	0.26
3	8.3	125	2977	24	23.8	3.79	2892	23.1	0.97	508	0.17
4	5.3	18.9	641	12.5	33.9	12.5	639	33.8	1.0	71	0.11
5	1.8	0.752	322	6.3	428	88.5	293	389	1.1	27	0.08

were specified as variable modifications. For protein identification, a minimum of two unique peptides was required. Peptides were accepted using a local false discovery rate (FDR) of <1.0%, as calculated by Proteome Discoverer (version 1.4; Thermo Fisher Scientific, San Jose, CA) from SequestHT by using a decoy protein database (reversed).

Criteria for Protein Identification—Scaffold (version Scaffold 4.3.2, Proteome Software Inc., Portland, OR) was used to validate MS/MS-based peptide and protein identifications. Peptide identifications were accepted if they could be established at >30.0% probability to achieve a FDR of <0.1%. Peptide probabilities from X!Tandem were assigned by the Peptide Prophet algorithm (37) with Scaffold delta-mass correction. Peptide probabilities from Sequest were assigned by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at >78.0% probability to achieve an FDR of <1.0% and contained at least two identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (38). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

RESULTS

Identification of GTL as KAT III/CCBL2—The glutamine analog, L-albizzin, is a better substrate of GTL than of GTK, and L-albizzin has been used to distinguish rat liver GTL from rat kidney GTK (6, 7, 9, 39). Thus, if, as we suspected from a comparison of the enzymatic properties and subunit composition of purified rat liver GTL (5) with those of purified recombinant mKAT III (22), the enzymes are products of homologous genes, then highly purified recombinant mKAT III should have considerable L-albizzin-glyoxylate transaminase activity. This was found to be the case. The recombinant purified mKAT III has a specific activity with this substrate pair of 1.85 units/mg.

To obtain further evidence that GTL is identical to KAT III, GTL was purified from mouse liver homogenates, and the specific activity of the enzyme using the L-albizzin-glyoxylate transaminase assay was compared with that of authentic highly purified recombinant mKAT III. The purification procedure employed was adapted from that of Cooper and Meister for the purification of GTL from rat liver (39). A summary of the purification procedure, specific activity, and yield is shown in Table 1.

The purified enzyme was subjected to SDS-PAGE and stained with Coomassie Blue (“Experimental Procedures”). Two major bands (M_r ~51,000 and ~37,000) and several smaller bands in the region M_r 40,000 to 50,000 were detected (data not shown). The M_r of mKAT III monomer is ~51,000 (22). Therefore, the upper band is consistent with the known M_r of mKAT III monomer. The pattern of bands indicated that the preparation of GTL purified from mouse liver was ~25% pure. The specific activity of this preparation was found to be 0.43 mmol/min/mg (units/mg). This value, assuming 25% purity, is consistent with the specific activity of the enzyme (L-albizzin-glyoxylate transaminase assay) found for the homogeneous recombinant mKAT III (1.85 units/mg).

Additional evidence that mGTL is identical to mKAT III was obtained by MS analysis. After SDS-PAGE, the stained band of M_r of ~51,000 (the calculated M_r of mKAT III monomer) was excised from the gel and processed for MS analyses as described under “Experimental Procedures.”

MS analysis revealed many peptides in the excised band assigned to 29 proteins (data not shown). Five proteins in the gel sample with the highest overall *p* value for likely correct identification were: 1) mitochondrial acetyl-CoA acetyltransferase, 2) 6-phosphogluconate dehydrogenase (decarboxylating), 3) cytosolic acetyl-CoA acetyltransferase, 4) aspartate aminotransferase 1 (cytosolic isozyme), and 5) KAT III. The sequences of the peptides found to highly correlate with mouse KAT III are listed in Table 2. KAT III was identified with 21 peptides, 19 of them unique to this protein (Δ score of 1) with a 54.15% amino acid sequence coverage.

Although our estimate that the glutamine transaminase isolated from mouse liver is only ~25% pure, it is highly likely that the L-albizzin/glyoxylate-, SM/ α -keto- γ -methiolbutyrate-, and MSC/ α -keto- γ -methiolbutyrate-transaminase activities (Table 3) associated with the preparation are due to KAT III and not to any other protein in the preparation. Enzymes 1, 2, and 3 (cited above) detected in the preparation do not possess aminotransferase activity. Enzyme 4 is an aminotransferase (cytosolic aspartate aminotransferase), but this enzyme is not known to have activity with glutamine or methionine. In fact, methionine is a very poor inhibitor of cytosolic aspartate aminotransferase with K_i of ~60 mM (40). Thus, it is

TABLE 2

Identification of mKAT III peptides in the GTL preparation purified from mouse liver

Modified residues are as follows: c, carbamidomethylcysteine; m, oxidized methionine (addition of one O); n, deamidated asparagine (Asn → Asp); q, deamidated glutamine (Gln → Glu). PSM, peptide spectrum match. A Δ score of 1.0 indicates that the peptide is unique to mKAT III. The X_{Corr} value is the cross-correlation value obtained from the search; a value of >2.0 indicates a good correlation. ΔM is the m/z difference between the designated peak and an adjacent peak. Missed cleavages are those “missed” during trypsin digestion.

Sequence	No. PSMs	Δ score	X_{Corr}	Charge	MH+	ΔM		No. missed cleavages
						Da	ppm	
HDTLcISDEVYEWLVYTGHTHVK	2	1.00	5.36	3	2802.34458	9.03	0	
LAADPSVVNLGQGFDPDISPPSYVK	13	1.00	5.22	2	2471.32622	17.98	0	
MDDPecYFNslPK	14	1.00	4.76	2	1615.70195	15.58	0	
RIEGLDSNVWVEFTK	3	1.00	4.71	2	1792.90752	-8.59	1	
IEGLDSnVWVEFTKLAADPSVVnLGQGFDPDISPPSYVK	2	0.02	4.68	3	4091.13352	19.49	1	
KLTAIPVSAFcDSK	3	1.00	4.66	2	1536.83599	17.48	1	
LAADPSVVnLGQGFDPDISPPSYVK	2	0.03	4.57	2	2472.28325	7.06	0	
KDSTLDAEEIFR	2	1.00	4.52	3	1494.76783	16.25	1	
mDDPecYFNslPK	16	1.00	4.47	2	1631.69792	16.08	0	
LAADPSVVNLGQGFDPDISPPSYVKEELSK	4	1.00	4.43	3	3057.60239	7.96	1	
IEGLDSNVWVEFTK	11	1.00	4.42	2	1636.85405	19.71	0	
LTAIPVSAFcDSK	32	1.00	4.30	2	1408.73467	14.57	0	
LLnSVGLKPIVPDGGYFIIADVSSLGADLSDmnSDEPYDYK	1	1.00	4.18	3	4406.10532	-2.46	0	
AAFIDNMnQYTR	12	0.02	3.97	2	1444.68059	19.38	0	
AAFIDNMnQYTR	18	1.00	3.95	2	1459.68987	18.07	0	
AAFIDNMnQYTR	27	1.00	3.94	2	1443.69011	14.91	0	
AAFIDNMnQYTR	6	0.01	3.83	2	1460.67583	19.39	0	
LGWSIGPAHLIK	1	1.00	3.80	3	1291.76630	10.93	0	
QELQVIADLcVK	20	1.00	3.70	2	1415.78435	19.78	0	
AILLNTPHNPLGK	5	1.00	3.40	2	1387.83098	18.24	0	
TKAILLNTPHNPLGK	1	1.00	3.33	2	1616.97991	19.53	1	
MAGAVPVFIPLR	1	1.00	3.27	2	1270.75688	17.92	0	
DSTLDAEEIFR	2	1.00	3.24	2	1366.66594	12.71	0	
WTSSDWTDFPR	4	1.00	3.24	2	1397.63555	16.77	0	
IEGLDSnVWVEFTK	1	1.00	3.22	2	1637			

TABLE 3

 β -Lyase activity of highly purified recombinant mGTL/KAT III toward various cysteine S-conjugates and β -chloro-D,L-alanine

Except in the case of β -chloro-D,L-alanine, the assay mixture (0.02 ml) contained 5 mM amino acid, 0.1 mM α -keto- γ -methiolbutyrate, and 100 mM potassium phosphate (pH 7.4). In the case of β -chloro-D,L-alanine, no α -keto- γ -methiolbutyrate was present in the assay mixture. After incubation at 37 °C for the time indicated, pyruvate was measured as indicated in the text. One unit of activity represents 1 μ mol of pyruvate formed per min ($n = 3$).

β -Lyase substrate	Incubation time	Enzyme added	Pyruvate formed	
	min	μ g	nmol	units/mg
TFEC	60	0.185	11.6 ± 0.8	1.045 ± 0.072
DCVC	60	0.185	6.00 ± 0.02	0.541 ± 0.002
BTC	120	0.74	<0.1	<0.001
Se-Methyl-L-selenocysteine	120	0.74	1.68 ± 0.30	0.019 ± 0.003
β -Chloro-D,L-alanine	60	0.185	5.13 ± 0.01	0.462 ± 0.001

unlikely that cytosolic aspartate aminotransferase in the preparation contributed to the transamination of L-albizzin (a glutamine analog) or to the transamination of SM/MS (methionine analogs). Of the other proteins in the preparation identified on the basis of peptide fragments, only one other aminotransferase was identified, namely mitochondrial GABA aminotransferase (data not shown). However, because of the relatively strict substrate specificity, it is unlikely that this enzyme contributed to the transamination of L-albizzin, SM, and MS. It is of note that the protein most closely related to KAT III (*i.e.* GTK/KAT I/CCBL1) was not detected in the KAT III preparation obtained from mouse liver.

Cysteine S-Conjugate β -Lyase Activity of Recombinant mKAT III and hKAT II—TFEC (5 mM) was shown to be a β -lyase substrate of highly purified recombinant mGTL/KAT III in the presence of 0.1 mM α -keto- γ -methiolbutyrate, indicating that this enzyme catalyzes a cysteine S-conjugate β -lyase reaction, as suggested by the gene designation *CCBL2* (Table 3). Because rates differ depending upon which substrate is used, both incu-

bation times and enzyme concentrations had to be varied. Despite differences in substrate, incubation times, and protein concentrations, the rate of formation of pyruvate in the presence of enzyme was linear for at least an hour under the conditions of the assay. This finding is important for two reasons. First, it permits enzyme rates to be calculated from fixed point determinations. Second, two reactive fragments (aminoacrylate and HSCF₂CF₂H) are produced from the β -lyase reaction with TFEC that can potentially lead to syncatalytic inactivation, *i.e.* occurring synchronously during catalysis. For example, rat liver mitAspAT catalyzes a β -lyase reaction with TFEC and is syncatalytically inactivated in the process (41). Covalent adduction of both aminoacrylate and reactive fluorine-containing fragments to the enzyme can be detected by mass spectroscopy (26). Although TFEC was not used, Stevens *et al.* (25) showed that rat GTK is not syncatalytically inactivated by DCVC during β -lyase turnover of this amino acid. Our studies show that human GTK is not inactivated by either DCVC or TFEC (27).

The present data suggest that the closely related mGTL is also not syncatalytically inactivated by TFEC during β -lyase reactions even after turnover of tens of thousands of substrate molecules in the lyase reaction. This contrasts with “susceptible” enzymes such as mitAspAT (41) and branched chain aminotransferase (42) that are inactivated on average following 50 to a few thousand turnover events (reviewed in Ref. 26).

Table 3 shows that, in addition to TFEC, recombinant mGTL/KAT III/CCBL2 possesses β -lyase activity toward DCVC, β -chloro-D,L-alanine, and MS. Under the conditions of the assay, β -lyase activity toward the various substrates is in the order: TFEC > DCVC \approx β -chloro-D,L-alanine > MS. Activity with BTC could not be detected. The specific activity with TFEC as a β -lyase substrate ($\sim 1.05 \mu$ mol/min/mg; pH 7.4) calculated

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from the data in Table 3 is only slightly less than that exhibited with L-albizzin as a transaminase substrate at pH 9.0 (1.85 $\mu\text{mol}/\text{min}/\text{mg}$). Thus, the enzyme coded by the gene *CCBL2/KAT III* possesses considerable cysteine S-conjugate β -lyase activity that, with a suitable β -lyase substrate (e.g. TFEC), is at least as comparable to its ability to catalyze transamination.

Experiments were performed to determine whether highly purified recombinant hAAT/KAT II can also catalyze β -lyase reactions similar to those catalyzed by mGTL/KAT III. Table 4 shows that this is indeed the case. The β -lyase activity of hKAT II toward various substrates is in the order: β -chloro-D,L-alanine \approx TFEC > DCVC > MSC. As noted for mGTL/KAT III, no activity could be detected with BTC. By contrast, some slow syncatalytic inactivation occurs with hKAT II, particularly when levels of pyruvate production in the assay mix exceed ~ 10 nmol.

TABLE 4

β -Lyase activity of AAT/hKAT II toward various cysteine S-conjugates and β -chloro-D,L-alanine

Except in the case of β -chloro-D,L-alanine, the assay mixture (0.02 ml) contained 5 mM amino acid, 0.1 mM α -ketoglutarate, 0.1 μg of hKAT II, and 100 mM potassium phosphate (pH 7.4). In the case of β -chloro-D,L-alanine, no α -ketoglutarate was present in the assay mixture. After incubation at 37 °C for the time indicated, pyruvate was measured as indicated in the text. One unit of activity represents 1 μmol of pyruvate formed per min ($n = 3$).

β -Lyase substrate	Incubation time	Pyruvate formed	
		min	nmol
TFEC	30	26.5 \pm 1.2	8.833 \pm 0.4
DCVC	30	4.66 \pm 0.18	1.553 \pm 0.006
BTC	120	<0.1	<0.008
Se-Methyl-L-selenocysteine	60	3.05 \pm 0.10	0.508 \pm 0.017
β -Chloro-D,L-alanine	15	15.0 \pm 0.8	10 \pm 0.053

TABLE 5

Comparison of substrate specificity of KAT enzymes and partially purified mouse liver GTL to transaminate L-selenomethionine and Se-methyl-L-selenocysteine to methionine

The reaction mixture (0.25 ml) contained 1 mM seleno-amino acid, 2 mM α -keto- γ -methiolbutyrate in 50 mM potassium phosphate buffer (pH 7.2). Each reaction mixture contained between 1 and 6 μg of the recombinant enzyme, and the reaction was incubated at 37 °C. At 15-min intervals, 0.05-ml aliquots were withdrawn, and the reaction was stopped with 0.015 ml of 25% metaphosphoric acid and cooled in ice for 10 min. L-Methionine was determined by HPLC with CoulArray detection. The values are the averages of four determinations.

	Specific activity	
	L-Selenomethionine	Se-Methyl-L-selenocysteine
	nmol/min/mg protein	
hKAT I/GTK/CCBL1	45 \pm 3	179 \pm 19
hKAT II/AAT	15 \pm 1	14 \pm 1
mKAT III/GTL/CCBL2	2153 \pm 259	163 \pm 11
Rat liver KAT IV/mitAspAT	<1	<1
GTL partially purified from mouse liver	389 \pm 15	36 \pm 6

TABLE 6

γ - and β -elimination reactions catalyzed by cystathionine γ -lyase

The reaction mixture (0.05 ml) contained amino acid substrate, enzyme, and 100 mM potassium phosphate buffer (pH 7.4). After incubation at 37 °C for the times shown, α -keto acid formation was determined by the 2,4-dinitrophenylhydrazine procedure.

Substrate	Concentration	Enzyme in assay mixture	Incubation time	Product measured	Product formed	
					mm	milliunits
L-Cystathionine	2	2.5	30	α -Ketobutyrate	8.40 \pm 0.12	269 \pm 4
	50	0.1	20	α -Ketobutyrate	1.83 \pm 0.05	2179 \pm 60
SM	20	2.5	40	α -Ketobutyrate	4.52 \pm 0.13	2690 \pm 77
			90		4.73 \pm 0.10	50 \pm 1
			180		9.58 \pm 0.20	51 \pm 1
MSC	10	2.5	10	Pyruvate	1.63 \pm 0.05	23 \pm 1
			20		2.83 \pm 0.10	27 \pm 1
			90		2.20 \pm 0.05	156 \pm 5
S-Allylmercapto-L-cysteine	2	2.5	180	Pyruvate	4.98 \pm 0.05	136 \pm 2

Identification of GTL/KAT III as a Major SM Aminotransferase in Mouse Liver—SM/ α -keto- γ -methiolbutyrate transaminase activity from mouse liver was found to co-purify with L-albizzin-glyoxylate transaminase activity at an essentially constant ratio of specific activities (Table 1). Inasmuch as L-albizzin transamination with glyoxylate and α -keto- γ -methiolbutyrate is catalyzed by GTL (6, 9, 39), the current findings are fully consistent with the hypothesis that GTL/KAT III in liver is a major enzyme responsible for catalyzing transamination of SM. On the other hand, the ratio of the rate of L-albizzin-glyoxylate transamination to that of MSC-glyoxylate transamination decreased by more than 90% during the purification (Table 1). This finding is consistent with GTK/KAT I also present in the liver that is a more effective catalyst for the transamination of MSC than it is for SM (Table 5).

Comparison of the Ability of Various KAT Enzymes to Catalyze Transamination of Seleno-Amino Acids—The ability of the various KAT enzymes to catalyze transamination between SM (and MSC) using α -keto- γ -methiolbutyrate as the co-substrate is shown in Table 5. Note that under the conditions of the assay, SM is a better substrate of highly purified recombinant mKAT III (GTL) than it is of KAT I (GTK), KAT II (AAT), and KAT IV (mitAspAT).

γ - and β -Elimination Reactions Catalyzed by Highly Purified Rat Liver Cystathionine γ -Lyase—Table 6 shows that rat liver cystathionine γ -lyase catalyzes a number of γ - and β -elimination reactions. Note that because of widely different relative activities, varying amounts of enzymes were used, and the time of incubation was also varied. The data illustrated in Table 6 show that the reaction is linear for the substrates investigated. Note also that L-homoserine is typically used in routine assays of cystathionine γ -lyase in place of L-cystathionine, because it is less expensive than the “natural” substrate L-cystathionine and, at saturating concentrations, is a more active substrate. It was previously reported that the K_m values exhibited by highly purified crystalline rat liver cystathionine γ -lyase toward L-homoserine and L-cystathionine are 20 and 3 mM, respectively (43). Moreover, the V_{max} was reported to be three times higher with L-homoserine than with L-cystathionine (43). The present findings with L-homoserine and L-cystathionine (Table 6) are consistent with this earlier report. In addition, we have verified the previous findings by Okuno *et al.* (29) that SM is a γ -lyase substrate of cystathionine γ -lyase.

It has long been known that cystathionine γ -lyase can catalyze β -elimination reactions with suitable substrates (e.g. cysteine, cystine) (44). We used *S*-allylmercapto-*L*-cysteine as a model β -lyase substrate of cystathionine γ -lyase. The relatively low, but easily detectable activity with *S*-allylmercapto-*L*-cysteine (Table 6) is consistent with our previous findings (34). We then determined whether MSC is a β -lyase substrate of cystathionine γ -lyase. Table 6 shows that MSC is indeed a β -lyase substrate of cystathionine γ -lyase. Although cystathionine γ -lyase is ostensibly a γ -lyase, it has activity with MSC as a β -lyase substrate under conditions of the assay (Table 6).

DISCUSSION

The present work describes some catalytic properties of mammalian KAT enzymes obtained from readily available sources. Despite the fact that these enzymes are derived from three different mammalian species, the present work has identified interesting findings applicable to mammals (including humans) in general.

Identification of GTL as KAT III/CCBL2—Several lines of evidence are presented here that GTL is identical to KAT III/CCBL2. The evidence includes previously published data on substrate specificity, pH optimum, subunit composition, and M_r . In the present work, we show that highly purified recombinant mKAT III has considerable activity with the glutamine analog *L*-albizziin, a previously known substrate of rat liver GTL (6, 8, 9, 39). Finally, mass spectral analysis provided additional evidence that GTL purified from mouse liver using *L*-albizziin as a substrate is identical to KAT III/CCBL2.

The broad specificity of many aminotransferases has created some confusion in nomenclature. For example, many workers refer to GTK as KAT I, despite the fact that glutamine is a much better substrate than is kynurenine (16). Moreover, glutamine is present in tissues in concentrations orders of magnitude higher than that of kynurenine. For example, the concentration of glutamine in most tissues, including the liver, is in the millimolar range, whereas the concentration of kynurenine in liver has been reported to be $\sim 0.4 \mu\text{M}$ (45). GTL was first described more than 40 years ago (5, 6, 8). By contrast, KAT III was first described only relatively recently (22, 23). Moreover, glutamine is a better substrate of KAT III than is kynurenine. Under conditions of the assays employed by Han *et al.* (22), the k_{cat}/K_m values for glutamine and kynurenine are 194 and $92 \text{ min}^{-1} \text{ mM}^{-1}$, respectively.

mGTL/KAT III/CCBL2 Is a Cysteine S-Conjugate β -Lyase—The present results show that, as inferred from the gene notation *CCBL2*, the mouse gene product (mGTL/KAT III/CCBL2) catalyzes effective β -lyase reactions with cysteine *S*-conjugates that contain a good nucleofuge in the β position. The enzyme also catalyzes a β -lyase reaction with β -chloro-*D,L*-alanine. The order of β -lyase reactivity (TFEC > DCVC \approx β -chloro-*D,L*-alanine > MSC; Table 3) is similar to that noted previously for its close homologs rat and human GTK (26). Rat kidney GTK (rGTK) exhibits little or no β -lyase activity toward BTC (Ref. 26 and references quoted therein). Similarly, the present work shows that mGTL/KAT III has no detectable β -lyase activity toward BTC. Additionally, we also showed that hKAT II/AAT has appreciable cysteine *S*-conjugate β -lyase activity with

TFEC, DCVC, β -chloro-*D,L*-alanine, and MSC but not with BTC (Table 4). Previously, we showed that BTC is a β -lyase substrate of rat liver mitAspAT, although it is less effective than is TFEC and DCVC (41). *In vivo* metabolites of BTC have previously been suggested to be markers of cysteine *S*-conjugate β -lyase activity (46). Although this suggestion is undoubtedly correct, the present study and previous work (47) show that the ability of BTC, compared with that of TFEC and DCVC, to act as a substrate of cysteine *S*-conjugate β -lyases may be limited especially with the KAT enzymes.

An interesting observation is that unlike some other PLP-containing enzymes that catalyze β -elimination reactions with TFEC and DCVC (e.g. mitAspAT), mGTL/KAT III/CCBL2 is not syncatalytically inactivated by these cysteine *S*-conjugates. hGTK/KAT I is also not syncatalytically inactivated by TFEC (26). This resistance of hGTK/KAT I and mGTL/KAT III/CCBL2 to inactivation may be due to the fact that there are no residues within the active site of either enzyme (e.g. cysteine or lysine; other than the lysine residue bound to PLP co-factor) susceptible to attack by aminoacrylate or by the eliminated sulfur-containing fragment.

The Scope of Cysteine S-Conjugate β -Lyases in Mammalian Tissues—Ten mammalian PLP-containing enzymes were previously shown to catalyze β -elimination reactions with cysteine *S*-conjugates derived from halogenated alkenes (26). Two of these enzymes are cystathionine γ -lyase and kynureninase. Eight of these enzymes are aminotransferases. Two other aminotransferases, namely GTL/KAT III (Table 3) and AAT/KAT II (Table 4) may now be added to this list. A β -elimination reaction with cysteine *S*-conjugates (or with β -chloro-*D,L*-alanine) is not a “natural” reaction catalyzed by these enzymes. These enzymes catalyze a β -elimination reaction when an amino acid with a strong leaving group in the β position binds to the active site and reacts with the PLP co-factor. In that case, electrons will be drawn toward the electronegative moiety ($-\text{SX}$ in $\text{XSCH}_2\text{CH}(\text{NH}_3^+)\text{CO}_2^-$; Equation 4), facilitating a β -elimination reaction that will compete with the transamination reaction. When the moiety in the β position is a strong electrophile, as is the case with β -chloro-*D,L*-alanine, β elimination predominates at the active site of mGTL/KAT III (and AAT-KAT II), and transamination cannot be detected. On the other hand, if $-\text{SX}$ has weak electron-withdrawing properties, then transamination will predominate over β elimination. Thus, *S*-methyl-*L*-cysteine is a transaminase substrate of rGTK, but not a β -lyase substrate (24, 27). On the other hand, rGTK and hGTK (24, 27) catalyze both transamination and β -elimination reactions approximately equally effectively with MSC. These findings are in accord with the fact that, because of the weaker nature of the Se-C bond relative to the S-C bond (48), $-\text{SeR}(\text{Ar})$ is a better nucleofuge than is $-\text{SR}(\text{Ar})$. The present work shows that mGTL/KAT III (and hKAT II), as noted for rGTK and hGTK, catalyzes competing transaminase and β -lyase reactions with MSC. The extent to which these current reactions occur *in vivo* will depend upon the tissue distribution or cellular content of cysteine *S*-conjugate β -lyases/aminotransferases.

Role of KAT Enzymes in Toxication of Halogenated Alkenes—A pathway for the metabolism of halogenated alkenes (e.g. trichloroethylene) involves conjugation with glutathione and sub-

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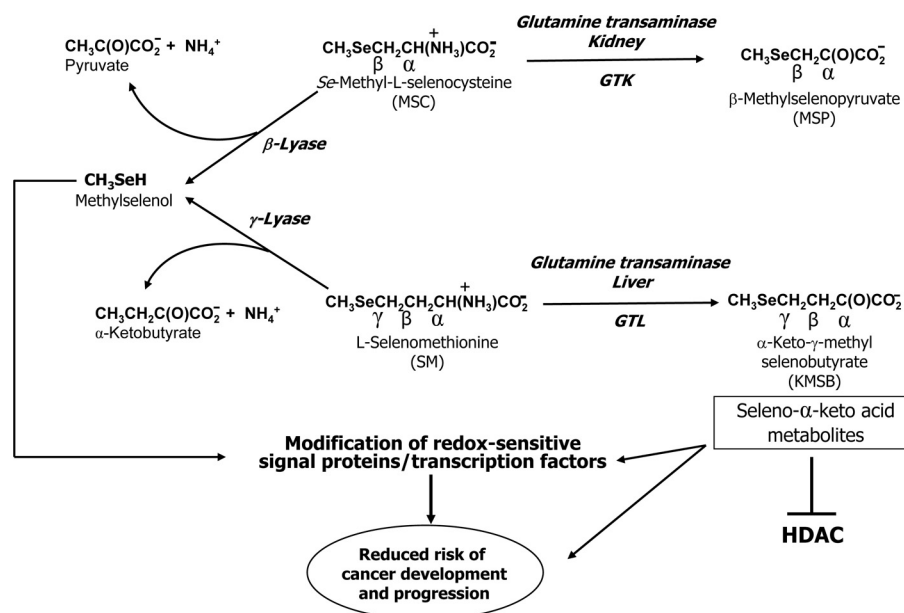


FIGURE 2. **Mechanisms for the chemopreventive properties of the major dietary seleno-amino acids.** GTK converts MSC to MSP, and GTL converts SM to KMSB. Both MSP and KMSB are potentially chemopreventive as HDAC inhibitors. In addition, methylselenol (CH_3SeH) and its oxidized form (methylseleninic acid, $\text{CH}_3\text{Se}(\text{O})\text{OH}$) are potentially chemopreventive via redox interactions with redox responsive signal proteins. Methylselenol is generated by a γ -lyase reaction catalyzed by cystathionine γ -lyase on SM. Methylselenol is also readily generated from MSC, because this amino acid is a β -lyase substrate of both cystathionine γ -lyase and GTL (and AAT). Cystathionine γ -lyase is present in mammalian tissues in high levels in liver and to a lesser extent in kidney and to a much smaller extent in other tissues. Moreover, we found that GTK activity was more prominent than that of GTL in various human prostate and colon cancer cell lines. Thus, MSC may serve as a better source of seleno- α -keto acids and methylselenol than does SM in certain human cancers. The use of SM as a dietary source of organoselenium rather than MSC may explain the failure of the SELECT study (see the text) (modified from Ref. 62).

sequent conversion of the glutathione *S*-conjugate to the corresponding cysteine *S*-conjugate (49–51). The cysteine *S*-conjugate β -lyase reaction bypasses the normal detoxification of the cysteine *S*-conjugate to the mercapturate and generates nephrotoxic thiol metabolites (50–54). Interestingly, not only are the cysteine *S*-conjugates of halogenated alkenes nephrotoxic, but even their precursor glutathione *S*-conjugates and corresponding mercapturates cause severe renal injury (55). The present work has extended the number of recognized mammalian cysteine *S*-conjugate β -lyases to include the KAT enzymes GTL/KAT III and AAT/KAT II.

Role of GTL/KAT III and GTK/KAT I in the Metabolism of Selenium-containing Amino Acids—Considerable epidemiological evidence suggests that diets rich in organoselenium compounds are chemoprotective (56, 57). In 1999, the SELECT study was undertaken to evaluate the effects of a selenium-enriched diet on human prostate cancer. Because vitamin E was included in one arm of the study, the acronym SELECT (Selenium and Vitamin E Cancer Prevention Trial) was chosen for the name of the trial. 32,000 men, ≥ 55 years of age were to be studied for a period of 12 years, and the results were originally scheduled for release in 2011. Early results (as of October 2008), however, were not encouraging (58). In 2008, the trial's data safety and monitoring committee recommended that participants discontinue taking the study supplements based on an interim finding of no preventive benefit (59). Because considerable epidemiological evidence suggests that organoselenium in the diet is chemopreventive, what could account for the contradictory findings of the SELECT study?

Major sources of organoselenium in the human diet include SM and more importantly MSC (60). Early in the SELECT

study, “selenized” yeast was chosen as the source of organoselenium. This dietary source is prepared by growing yeast in a broth containing inorganic selenite which organifies selenium into metabolites previously dominated by sulfur such as glutathione, methionine, cystine, and cysteine. Later in the SELECT study, this mixture of organoselenium was switched directly to SM.

Our studies suggest that use of SM as the sole dietary source of organoselenium rather than MSC could account for the apparent discrepancy in findings of the SELECT study *versus* that of other clinical trials which used selenium enriched food sources (61). We previously showed that the seleno- α -keto acid analogs of both SM (*i.e.* KMSB) and MSC (*i.e.* MSP) are HDAC inhibitors ($K_i = 10\text{--}20\ \mu\text{M}$) in human prostate and colon cancer cells in culture (30, 62, 63). However, MSC, but not SM, elicited HDAC inhibition in these cells. We have found that extracts of human prostate cancer cells have no detectable ability to transaminate SM to KMSB (62). Nor do they exhibit the ability to convert SM to α -ketobutyrate (and by inference, no ability to form methylselenol from this amino acid). Because GTL/KAT III exhibits strong activity toward SM (Table 5), the finding that the presence of SM does not elicit an HDAC inhibitory response in cultures of human cancerous prostate cells and in cancerous colon cells may be explained by the absence of GTL/KAT III in these cells. On the other hand, we have shown that several human cancer cell lines, including prostate and colon cancers, have GTK/KAT I activity (62, 63). Thus, these cells are able to convert MSC to the HDAC inhibitor, MSP.

One other factor possibly contributing to the negative findings of the SELECT study should be mentioned. As discussed above, Okuno *et al.* (29) have shown that SM is a substrate of

mouse liver cystathionine γ -lyase. The products of this reaction are α -ketobutyrate, ammonia, and methylselenol. However, Suzuki *et al.* (64) were unable to detect γ -elimination of SM from a rat liver homogenate (a rich source of cystathionine- γ -lyase). Nevertheless, in terms of α -ketobutyrate production, we have verified the findings of Okuno *et al.* for the rat enzyme (Table 6). Interestingly, Suzuki *et al.* were able to show that rat liver homogenates efficiently convert MSC to pyruvate, ammonia, and selenite (presumably arising from the demethylation of methylselenol) (65). Suzuki and colleagues have provided a strong case that methylselenol and its oxidized form, methylseleninic acid, are chemopreventive by altering the status of redox responsive signal proteins (64–66). These authors have concluded that MSC is not only a selenium supplement but also a more promising precursor of the antitumor form of organoselenium than is SM, which is currently used in over the counter selenium supplements (66).

Based on the present findings, the β -lyase activity toward MSC is catalyzed in rat liver by GTL/KAT III and GTK/KAT I, both of which are present in relatively high amounts in this organ (8). We have shown that, under the conditions of our assay, MSC is a relatively good β -lyase substrate of purified rat liver cystathionine γ -lyase (Table 6). The specific activity of cystathionine γ -lyase in the rat is highest in the liver followed by the kidney (43). There is little activity in other tissues (43). Thus, *in vivo*, the liver is expected to be especially active in converting MSC to methylselenol and MSP. Similarly, SM was also shown to be a γ -lyase substrate of cystathionine γ -lyase, although less effective than MSC as a β -lyase substrate (Table 6). SM is also a good substrate of GTL. Thus, the liver is expected to be able to effectively convert SM to methylselenol and KMSB.

We have investigated the enzymatic profiles of several prostate and colon cancer cell types (62, 63). In all cases, GTK/KAT I activity could be detected, but not cystathionine γ -lyase or only marginally, GTL/KAT III activity. Thus, MSC, and not SM, may provide a more promising source of chemopreventive efficacy associated with methylselenol and the seleno- α -keto acid, MSP. This enzyme profile provides an explanation for the apparent lack of effect of SM in the SELECT study and provides a rational basis for the statement from the Suzuki group that MSC may be a better chemopreventive agent than is SM. However, it is possible that SM will be chemopreventive in tumors derived from the liver or splanchnic tissues, if they retain GTL and cystathionine γ -lyase activities. Fig. 2 summarizes metabolic conversions of both MSC and SM into metabolites that exhibit promise for cancer prevention and control.

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Kynurenine Aminotransferase III and Glutamine Transaminase L Are Identical Enzymes that have Cysteine S-Conjugate β -Lyase Activity and Can Transaminate L-Selenomethionine

John T. Pinto, Boris F. Krasnikov, Steven Alcutt, Melanie E. Jones, Thambi Dorai, Maria T. Villar, Antonio Artigues, Jianyong Li and Arthur J. L. Cooper

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