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HUMAN HEPATIC C-S LYASE: TRANSAMINATION REACTIONS AND SIGNIFICANT DIFFERENCES BETWEEN KYNURENINE AMINOTRANSFERASE AND KYNURENINASE

Lorraine D. Buckberry, Barrie W. Bycroft, P. Nicholas Shaw

Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD

and

Ian S. Blagbrough*

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

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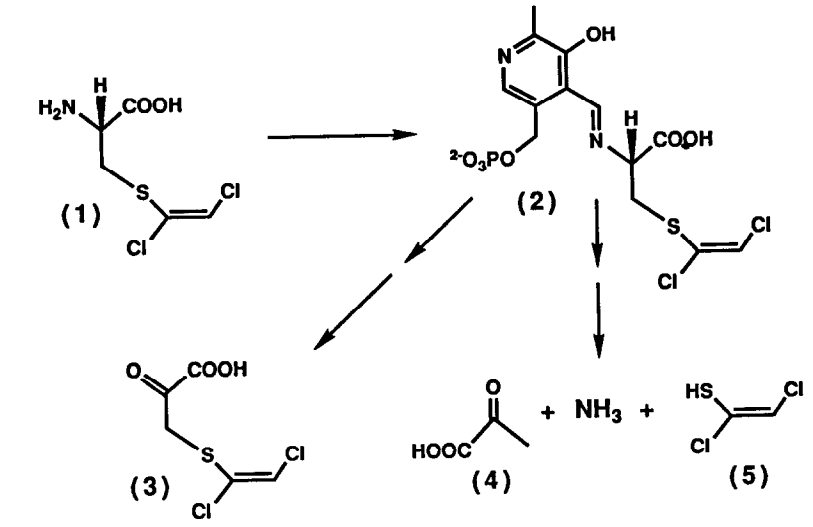
Abstract: Kynurenine aminotransferase and glutamine transaminase K and L activities were assessed in order to identify a physiological role for human hepatic cytosolic C-S lyase (CSL), as the C-C lyase kynureninase, which co-purifies with rat hepatic CSL, has been shown not to be human hepatic CSL. Kynurenic acid production supports an aminotransferase role for this CSL.

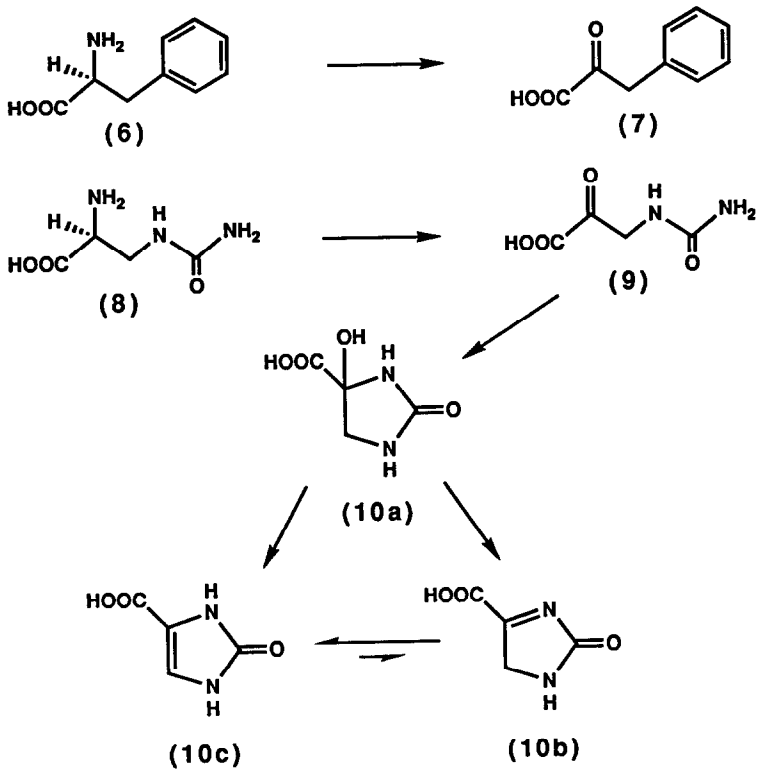
Many xenobiotics undergo conjugation reactions with the ubiquitous tripeptide glutathione, bond-forming reactions occurring at the nucleophilic thiol group of the cysteine residue either with or occasionally without catalysis by the enzyme glutathione *S*-transferase. Glutathione conjugates then undergo further metabolism by any of a large number of pathways to afford various highly polar and water-soluble metabolites, the most important of which, in mammals, are the mercapturic acids (*N*-acetyl-L-cysteines) which are excreted in urine or bile. The pathway leading to mercapturates has been generally regarded as detoxifying. C-S lyase (CSL) enzymes are responsible for the generation of toxicity as a result of lysis of the C-S bond in the L-cysteine conjugates of certain halocarbon xenobiotics. Such lysis generates reactive, cytotoxic, and/or mutagenic thiols [1] (Scheme 1). In this *Letter*, we report the results of our studies on transamination reactions and we highlight the significant differences between kynurenine aminotransferase and kynureninase. We also report the results of our experiments using human hepatic tissues in order to identify CSL activity with *S*-(*E*-1,2-dichlorovinyl)-L-cysteine (DCVC) (**1**) as the substrate [2], and we raise the issue of the physiological role(s) of hepatic CSL enzymes.

DCVC (**1**) (17.2 mM) (the experimentally determined saturating substrate concentration for human hepatic CSL is 14 mM) [2, 3] was used to determine CSL activity by monitoring the production of pyruvic acid (**4**) [4] at 37°C and pH = 7.5. The mechanism of the reaction is an α-β elimination from the corresponding Schiff base (**2**) with transamination to (**3**), but also β-lysis to pyruvic acid (**4**) and, in equal stoichiometry, to a reactive, toxic enethiol (**5**) (Scheme 1). Cysteine conjugate CSL activity was detected in all the sub-cellular fractions of human hepatic tissues from one male (25 years old) and two female (30 and 90 years old) donors [2]. The homogenates contained a total activity/g tissue of 172 with the male, 220 with the older female, and 121 with the younger female subject. The enzyme specific activities (SA) of the homogenates from the male and older female tissues were not significantly different (1.58±0.27 and 1.47±0.02 mUnit/mg respectively), however the homogenate of the younger female had a lower SA (0.95±0.01 mUnit/mg protein where 1 Unit is defined as 1 μmol pyruvate produced/min of incubation). The cytosolic to mitochondrial SA ratios were significantly different at 0.67 and 3.35 for the younger and older females respectively. The total activity/g tissue in the younger female was 0.55 times that in the cytosol of the older. The SA of the mitochondrial fraction of the older female was only 30% that of the cytosolic, however, in the younger female donor, the mitochondrial activity was 150% that of the cytosol.

We have investigated the physiological role of human hepatic cytosolic CSL by assaying glutamine transaminase K (GTK), glutamine transaminase L (GTL), and kynurenine aminotransferase (KAT) activity during the CSL purification [5]. Transaminase activity towards L-phenylalanine (**6**) and L-albizziin (**8**), an analogue of glutamine (**13**) in which a carbamate isosterically replaces the primary amide, is referred to as L-phenylalanine aminotransferase (PAT) and L-albizziin aminotransferase (AAT) activity respectively. GTK and GTL enzymes have overlapping substrate profiles, both activities are found in liver and kidney [6 (a-d)]. GTK has (**6**) and GTL has (**8**) as specific substrates affording phenylpyruvic acid (**7**) and 2-imidazolone-4-carboxylic acid (**10c**) respectively, *via* transamination to (**9**) and then cyclization to 4-hydroxy-2-imidazolidinone-4-carboxylic acid (**10a**), with facile dehydration of the cyclic urea to (**10c**) [6 (c)], or possibly *via* the imine (**10b**) and tautomerism to the enamine (**10c**). PAT therefore reflects GTK activity, just as AAT is an established measure of GTL activity [6 (a-d)]. Kynurenine (**17**) was used as the substrate of choice to determine KAT activity [7]. Products (**7**), (**10c**), and (**23d**) have intense, selective UV absorption maxima which are amenable to quantitative analysis. Amino acids (**6**), (**8**), and (**17**) were transaminated with the initial sub-cellular preparations, but PAT and AAT SA diminished during the purification of human hepatic CSL [5]. During this purification, however, KAT activity was shown to increase significantly.

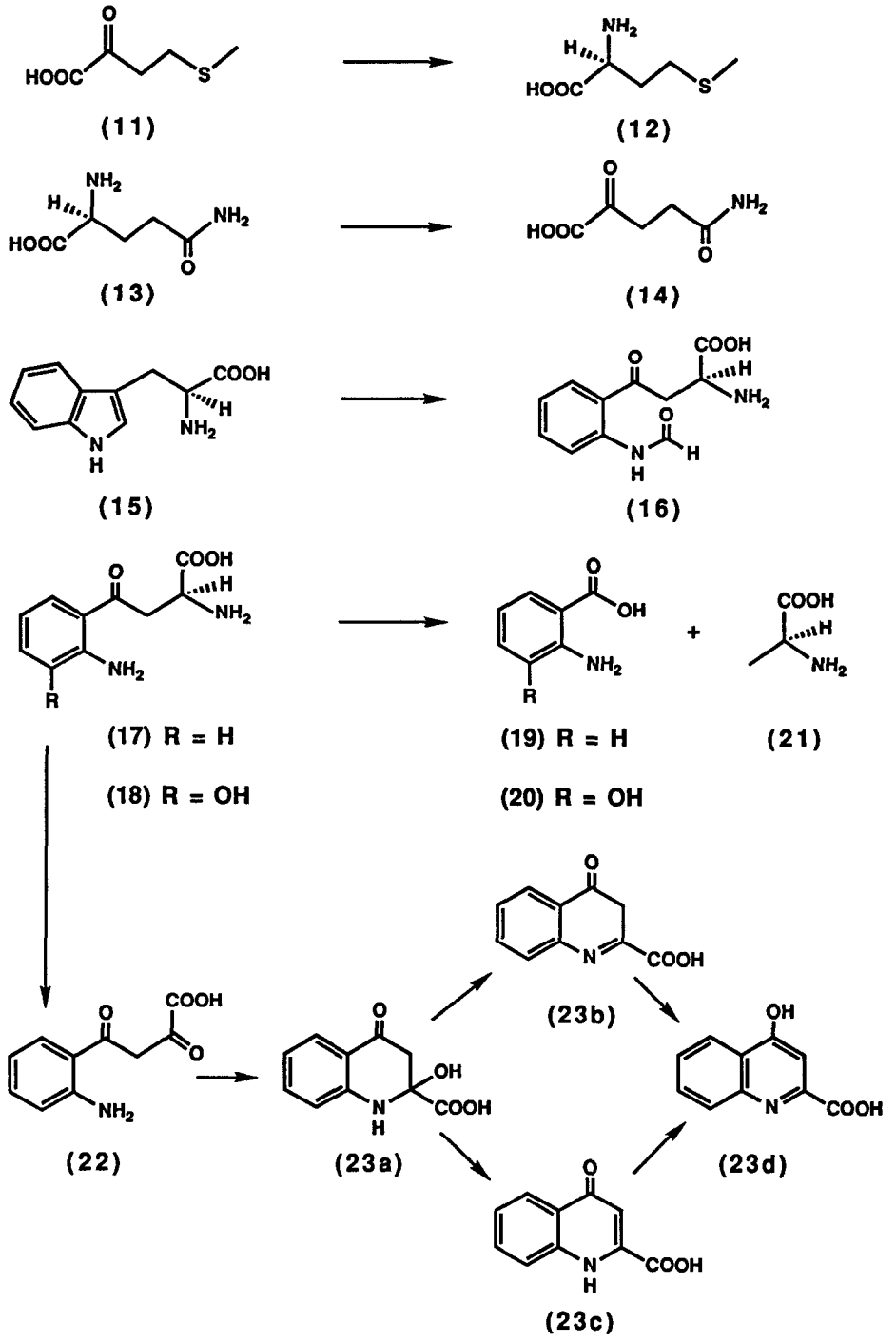


Scheme 1



Enzymes capable of C-S bond lysis have been identified and purified from plants, bacteria, and animal tissues [8]. CSL activity has been shown to be a property of various transaminase enzymes. The relationship between lysis from the aldimine competing with ketimine formation has also been observed with glutamic acid decarboxylase [9]. In rat tissue, GTK activity is widely distributed, not confined to the kidney, but also found in the liver and brain [6 (d)]. The research groups of Stevens [10], Gibson [11], and, independently, our own studies [12] have established, albeit only from the size, sub-unit composition, and substrate profile, that rat renal cytosolic CSL is identical to the soluble form of GTK [6 (a-d)]. The tissue distributions of CSL and GTK activity in the rat have also been determined: CSL and GTK activities were highest in the kidney [13]. Renal CSL activity was stimulated by the addition of α-keto-γ-methiolbutanoic acid (**11**) which afforded L-methionine (**12**) coupled with the transamination of glutamine (**13**) to the α-keto acid (**14**). In human tissue, renal CSL also co-purifies with GTK activity [14, 15].

L-Tryptophan (**15**) is metabolized by ring scission to *N*-formyl kynurenine (**16**), subsequent decarbonylation affords kynurenine (**17**). 3-Hydroxy-kynurenine (**18**) and (**17**) are substrates for rat hepatic cytosolic CSL, but producing L-alanine (**21**) by C-C bond cleavage (hydrolysis) and not pyruvic acid from C-S bond lysis [16]. Kynureninase catalyses the hydrolysis of (**17**) and (**18**) producing anthranilic acids (**19**) and (**20**) respectively in equal stoichiometry with (**21**). The co-purification of kynureninase and rat hepatic CSL activity led to the proposal that rat hepatic CSL is the C-C lyase kynureninase (EC 3.7.1.3) converting (**15**) into biosynthetic precursors of nicotinamide ribonucleotides [16]. The physiological role of human hepatic CSL is not that of kynureninase, as the enzyme does not cleave kynurenine (**17**) to anthranilic acid (**19**) and L-alanine (**21**) [17]. We propose that its role is that of kynurenine aminotransferase converting (**17**) into (**22**), followed by cyclization to (**23a**), dehydration to the imine (**23b**) or the vinylogous amide (**23c**), and then tautomerism to kynurenic acid (**23d**). We have demonstrated that KAT activity co-purifies with cysteine conjugate CSL activity from both male and female donors [5]. Two isoenzymes of KAT have been detected in human liver [18], with pI's of 8.0 and 5.0. Each enzyme comprises two identical sub-units of dimeric M Wt 90kDa. Our data [5] for human hepatic CSL isoenzymes (including M Wt and isoelectric points) are in good agreement with those reported for human hepatic KAT [18] and are also comparable with those of rat renal KAT which catalyses the transamination of 3,5-diiodo-L-tyrosine [19, 20, 21]. Therefore, one function of this enzyme might be thyroid hormone metabolism [7, 19, 21] and some further support for this proposal comes with the observation that aromatic cysteine conjugates are substrates for human hepatic CSL [2, 17].


(11)
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(17) R = H
(18) R = OH
(19) R = H
(20) R = OH
(21)
(22)
(23a)
(23b)
(23c)
(23d)

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