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KYNURENINE AMINOTRANSFERASE/HUMAN HEPATIC C-S LYASE: PRELIMINARY STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

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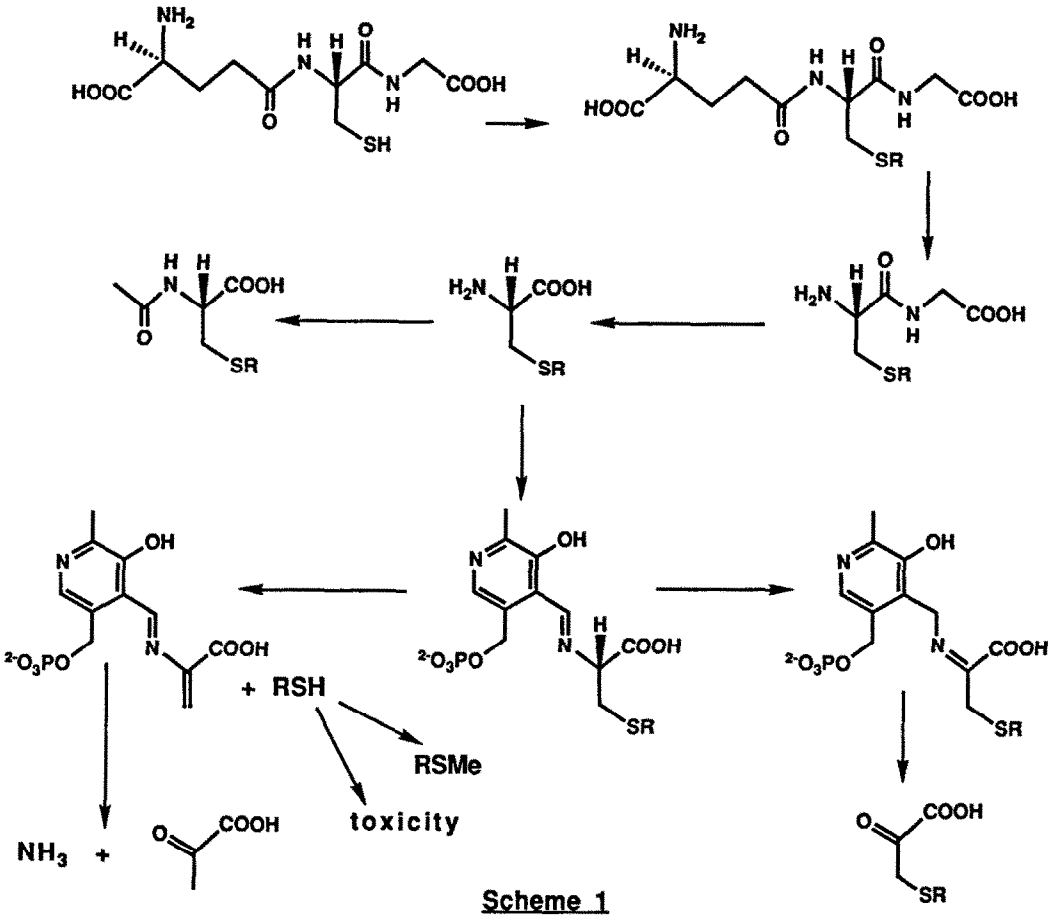
Abstract: Partially purified human hepatic cytosolic and mitochondrial fractions have been investigated for evidence of C-S lyase (CSL) activity. CSL activity has been characterized with synthetic aliphatic and aromatic L-cysteine conjugates. Preliminary structure-activity relationship studies have shown that aliphatic and aromatic L-cysteine conjugates are substrates.

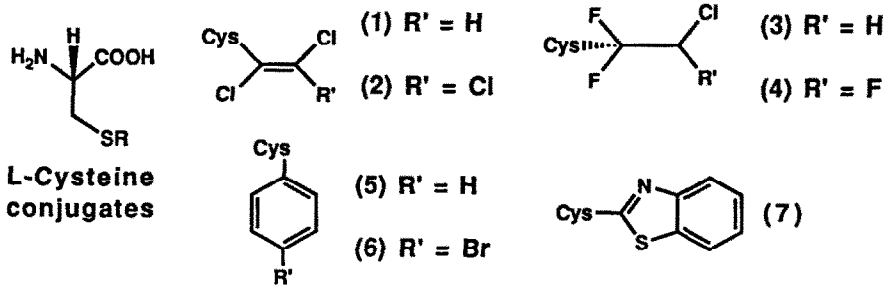
The mercapturic acid pathway has been generally regarded, until recently, as detoxifying. However, C-S lyase (CSL) enzymes are responsible for the generation of toxic sequelae as a result of the processing by these enzymes of the L-cysteine conjugates of halocarbon xenobiotics. These cysteine conjugates are biosynthetic intermediates in the normal mercapturic acid pathway in mammals (see Scheme 1). Thus, toxic thiols may result from a detoxification pathway. The pyridoxal phosphate dependent CSL enzymes, which facilitate this α-β elimination reaction on L-cysteine conjugate metabolites, are generally dimers of M Wt approx. 100 kDa. The products of the CSL catalysis of L-cysteine conjugates are, in equal stoichiometry, thiols, pyruvic acid, and ammonia (Scheme 1). In this *Letter*, we present the results of experiments to characterize mammalian CSL activity in human hepatic cytosolic and mitochondrial fractions. Our current research into the structure-activity relationships (SAR) of CSL enzymes [1, 2, 3, 4] is focused upon the aberrant processing of glutathione conjugates by CSL which has the potential to afford thiols which are cytotoxic and/or mutagenic and which may themselves be methylated (see Scheme 1) {for recent examples of CSL SAR and mode of action studies, see, *inter alia*: [1, 2, 3, 4, 5 (a-i)]}.

Liver was obtained *post-mortem* from a Caucasian male (25 years old) who died as a result of a cardiac arrest, a Polynesian pregnant female (30 years old) who died after a brain haemorrhage, and a Caucasian female (90 years old) who died after multiple fractures. In all cases, the liver was removed within 4 h of death, cubed, washed in aqueous potassium chloride solution (0.9% w/v) at 4°C, and frozen at -80°C until required. Enzyme activity (mUnit/mg protein, 1 Unit is defined as 1 μmol pyruvate produced/min of incubation) and SAR were determined using the mitochondrial fraction of the homogenate, and an ammonium sulphate precipitated fraction (25-40% cut) of the cytosol. Pyruvate levels were determined by monitoring NADH turnover at 340 nm [6] and protein levels were standardized against aqueous bovine serum albumin solution [7]. A homogeneous protein (monitored by SDS-PAGE with Coomassie Brilliant Blue R-250 or more sensitive silver staining, 12% gels, 0.5 to 1.0 mm thick) was obtained following purification [8] with an 11-fold increase in CSL enzyme specific activity (SA) observed to a final SA of 15.3 mUnit/mg protein. The molecular weight of the enzyme was 37 kDa in these denaturing conditions, 83 kDa non-denaturing (CHAPS), and the enzyme was shown to co-purify with kynurenine aminotransferase activity, an enzyme quite distinct from kynureninase [9]. L-Cysteine conjugates were prepared [10, 11, 12, 13], typical mercapturic acid precursors: chloroalkenes (**1**) and (**2**), haloalkyls (**3**) and (**4**), aromatic compounds (**5**) and (**6**), and an aromatic heterocycle (**7**). Satisfactory spectroscopic (ir, uv, [α], and ms), chromatographic (homogeneous by tlc), and analytical (literature mp) data were obtained for: *S*-(*E*-1,2-dichloroethenyl)- [formerly *S*-(*E*-1,2-dichlorovinyl- (DCVC) (**1**)], *S*-(1,1,2-trichloroethenyl)- (**2**), *S*-(2-chloro-1,1-difluoroethyl)- (**3**), *S*-(2-chloro-1,1,2-trifluoroethyl)- (**4**), *S*-(phenyl)- (**5**), *S*-(4-bromophenyl)- (**6**), and *S*-(2-benzothiazolyl)-L-cysteine (**7**).

The best substrates when incubated at pH = 8.5 and 37°C with the cytosolic fraction were (**1**) and (**2**) (6.2 and 5.6 mUnit/mg respectively), assayed only at a saturating substrate concentration (16mM). However, (**1**) and (**3**) were the most active substrates with the mitochondrial fraction (3.2 and 2.7 mUnit/mg respectively). The saturated aliphatic substrates displayed only half the activity of the unsaturated substrates and the aromatic substrates less than 10% of the activity of (**1**) or (**2**) when assayed in the cytosolic fraction. The mitochondrial preparation displayed only half the activity (3.2±0.8 mUnit/mg) that had been observed with the cytosolic preparation (6.2±0.5 mUnit/mg) using DCVC (**1**) as the substrate. Greater activity was found with (**3**) (2.7±0.2 mUnit/mg) in the mitochondrial fraction than with (**2**) (2.0±0.2 mUnit/mg). The aliphatic conjugates (**1**)-(**4**) were significantly better substrates than the aromatic conjugates tested in mitochondrial or cytosolic fractions (see histogram). Aromatic conjugates (**5**), (**6**), and (**7**) were more active with the mitochondrial preparation than with the cytosolic.


+ RSH
RSMe
toxicity
NH3 +

Scheme 1


L-Cysteine conjugates
(1) R' = H
(2) R' = Cl
(3) R' = H
(4) R' = F
(5) R' = H
(6) R' = Br
(7)

We have characterized human hepatic CSL with pH stability and temperature profiles. The pH profiles of CSL activity in the human hepatic cytosolic and mitochondrial fractions were examined with DCVC (**1**). The pH range chosen was 5.5 to 8.5 and aqueous potassium phosphate buffer (50 mM) was used to ensure pH stability. In the cytosolic preparation, the CSL SA was optimal at pH = 6.5 and remained at this level (approx. 6.0 mUnit/mg) up to pH = 8.5. The optimal activity (approx. 3.5 mUnit/mg) of the mitochondrial fraction was observed between pH 7.0 and 8.0. When the liver of a human male was examined using (**6**) the optimum pH was 8.5 in Tris buffer [14]. The SA of the cytosolic and mitochondrial preparations increased with incubation temperature up to 57°C, however the extent of this temperature dependent increase diminished at temperatures above 37°C. There was no observable loss of CSL activity due to storage at -20°C over 2 weeks. After extended periods, the stability of the enzyme was enhanced by the addition of pyridoxal phosphate (20 μM). On repeated freeze/thawing, human hepatic CSL activity decreased by between 20 and 50%. We have also demonstrated that human hepatic CSL enzymes are more thermostable than the equivalent human renal CSL enzymes which were denatured above 47°C [15].

In this *Letter*, we have reported the observation of cysteine conjugate CSL activity in the sub-cellular fractions of human hepatic tissue. The data show that DCVC (**1**) is the optimal substrate for the assay of CSL activity in human hepatic enzymes [3]. It has previously been proposed that the rat hepatic CSL enzyme has two broad substrate requirements [16]: (i) the substrate must be a conjugate of L-cysteine, not of glutathione or *N*-acetyl cysteine; (ii) the substrate must possess an aromatic moiety. Not unexpectedly, as the lysis is enzyme catalysed, the former stereospecific requirement also applies to human hepatic CSL, however, the latter substituent constraints do not apply in this instance as (**1**), (**2**), (**3**), and (**4**) are substrates. Therefore, a degree of restraint should be cautioned if extrapolating biotransformation results from animal model experiments to human toxicology with respect to the CSL mediated pathway of toxification by aberrant glutathione conjugate metabolism. Furthermore, any compound which is not a renal toxin in an animal model may still be a substrate for human CSL (renal or hepatic) with obvious implications for environmental and occupational exposure to xenobiotics that are detoxified mainly by the mercapturic acid pathway.

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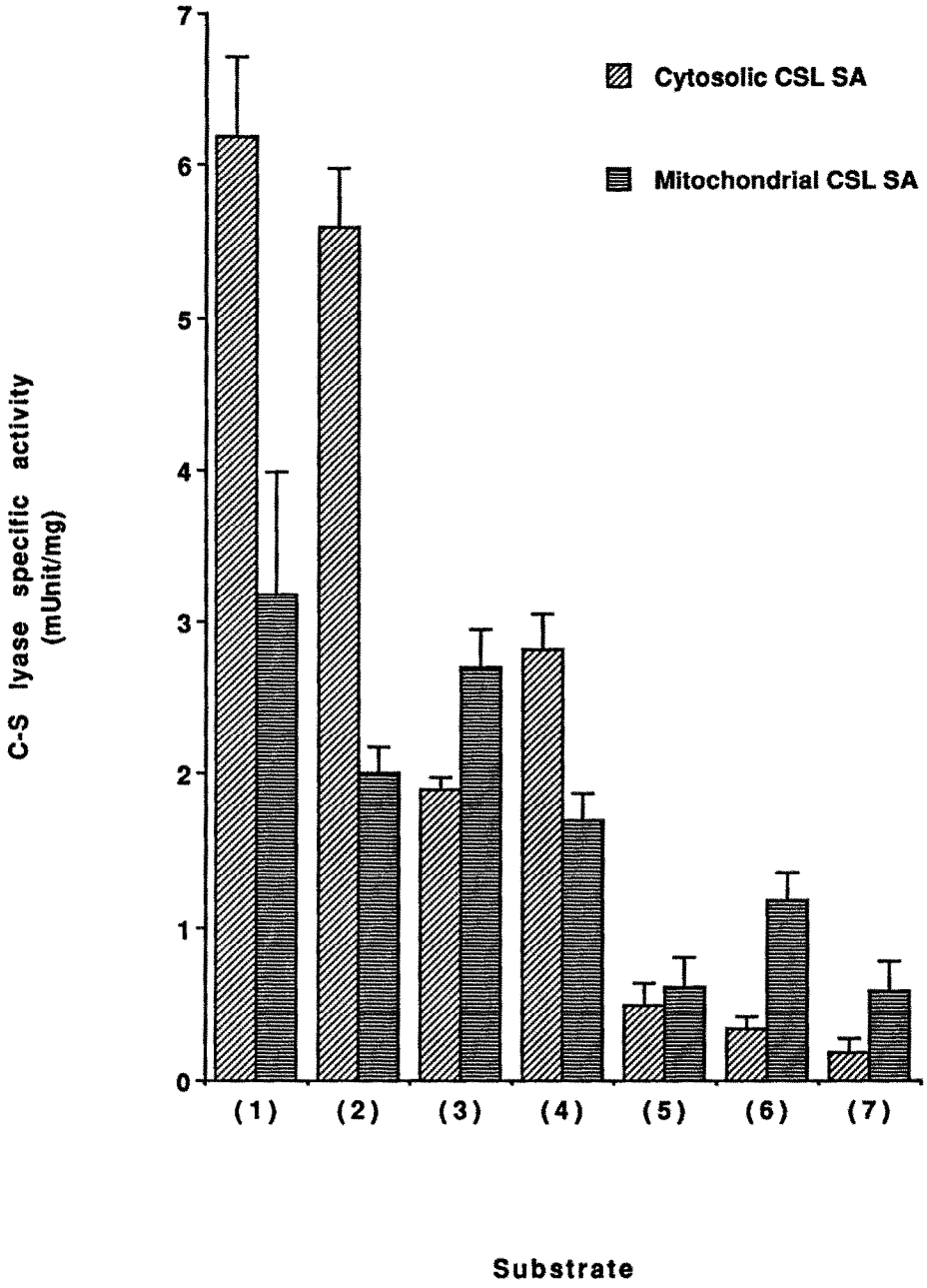
Cytosolic CSL SA
Mitochondrial CSL SA
C-S lyase specific activity (mUnit/mg)
0
1
2
3
4
5
6
7
(1)
(2)
(3)
(4)
(5)
(6)
(7)
Substrate

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