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HUMAN HEPATIC C-S LYASE: CO-PURIFICATION WITH KYNURENINE AMINO TRANSFERASE

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The C-S lyase enzymes are responsible for the generation of mutagenic and cytotoxic metabolites via aberrant drug metabolising pathways in mammalian tissues. We have examined human hepatic cytosolic and mitochondrial fractions for evidence of C-S lyase activity.

S-(E-1,2-Dichlorovinyl)-L-cysteine was used as the substrate (10mM) and enzyme activity was determined by assaying for pyruvic acid. C-S lyase specific activity (nmol/min/mg) in cytosol (2.55±0.20) was 3-fold greater than that observed in mitochondria (0.85±0.30) and enzyme activity did not increase on the addition of sodium α-keto-γ-methiolbutyrate (KMB). The co-incubation with pyridoxal phosphate resulted in a marked increase in the mitochondrial specific activity (3.15±0.20), but in only a minor improvement in that of the cytosolic enzyme (3.20±0.30). The optimum pH for enzyme activity, in both fractions, was 7.5. The cytosolic enzyme was purified using FPLC over FFQ Sepharose, Mono P (chromatofocusing), and Superose 12. This produced a homogeneous protein (monitored by SDS-PAGE) with a concomitant 15-fold increase in specific activity. At each stage of the lyase purification, a number of enzyme activities were studied in an attempt to identify a physiological role for the lyase enzyme. Kynurenine aminotransferase activity was quantified using kynurenine as the major substrate with α-ketoglutarate as the co-substrate. The product, kynurenate, was quantified spectrophotometrically at 333nm. Glutamine transaminase K activity was determined using phenylalanine as the major substrate and KMB as the co-substrate. The concentration of the product of the transamination reaction, phenylpyruvate, was determined spectrophotometrically at 322nm. Glutamine transaminase L activity was determined using albizziin as the major substrate and KMB as the co-substrate. The product of the transamination, 2-imidazolinone-4-carboxylic acid was quantified at 280nm.

Other workers have reported that human and rat renal C-S lyase preparations co-purify with glutamine transaminase K activity (Stevens et al 1986; Lash et al 1990). It has also been shown that rat hepatic C-S lyase does not co-purify with glutamine transaminase L, but with the C-C lyase kynureninase (Stevens and Jakoby, 1985). Tomisawa et al (1986) have reported that human hepatic C-S lyase did not co-purify with kynureninase activity. Human hepatic C-S lyase does not co-purify with either glutamine transaminase K or L activity. However, it does co-purify with kynurenine aminotransferase. Kynurenine aminotransferase also co-purifies with halogenated tyrosine aminotransferase (Tobes and Mason, 1978). Two isoenzymes of kynurenine aminotransferase have been reported to be present in human liver. The enzyme has pI's of 5.0 and 8.0, and consists of a dimer (90kDa), with a monomeric weight of 45kDa (Kido, 1984). These results are consistent with our own data regarding the molecular weight and sub-unit composition of human hepatic cytosolic C-S lyase.

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