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CYTOSOLIC SULPHOXIDATION OF S-CARBOXYMETHYL-L-CYSTEINE IN MAMMALS

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INTRODUCTION

The formation of xenobiotic sulphoxides from sulphide precursors has generally been assumed to be undertaken by enzyme systems located within the microsomal fraction of cell preparations - associated with the endoplasmic reticulum of the intact living cell. Such systems have included the ubiquitous cytochrome P-450 family of enzymes and the P-450 independent flavin-containing monooxygenase, together with others such as prostaglandin synthetase which may contribute in part to oxygenation of some sulphides (1,2). It is becoming apparent that such a multiplicity of enzyme systems is necessary because although we may be aware of only one type of sulphur the cell undoubtably recognises several different sulphurs dependent upon the surrounding electromolecular environment of that particular atom, the substrate choosing which enzyme system is best suited to oxidise its own particular sulphur, with a certain degree of overlap usually occurring (3).

Evidence has been accumulating that cytosolic systems may also be involved in these reactions, albeit perhaps non-specific, and recent observations in our laboratories have suggested that the sulphoxidation of the xenobiotic mucolytic agent, S-carboxymethyl-L-cysteine ('Mucodyne', Berk Pharmaceuticals, UK), may be cytosolic and not microsomal in nature (4,5). This proposition has now been investigated in a variety of species.

METHODS

Livers were obtained from the following adult animals; rats (Wistar), guinea pigs (Dunkin-Hartley), rabbits (New Zealand White), hamsters (Syrian) and sheep (Hampshire Cluns). Human livers were obtained from a liver transplantation programme. Cell fractions were prepared by the standard differential centrifugation technique (6), all procedures being carried out at 0-4°C and over ice where possible. Briefly, washed liver (10g) was homogenised (Potter-Elvehjem) for one minute in 0.1M potassium phosphate buffer, pH 7.4 (50ml) and then centrifuged for thirty minutes at 10,000g. The pellet (cell debris, nuclei and mitochondria) was discarded and the supernatant centrifuged at 100,000g for one hour. The supernatant (cytosol) was removed for incubation whilst the pellet was resuspended in buffer (30ml) and centrifuged again for one hour at 100,000g. The resultant supernatant was discarded and the pellet (washed microsomes) resuspended in buffer (30ml). Cross contamination of the fractions was examined by the use of enzyme markers; lactate dehydrogenase for the cytosol (Sigma Diagnostics, Dorset, UK) and cytochrome P-450, measured as its CO-complex (7), for the microsomes. Protein concentration was determined by the biuret method (8), bovine serum albumin being employed as a standard (Sigma Diagnostics).

A typical incubation mixture consisted of 5ml cytosol (100,000g supernatant) or microsome suspension previously gassed for 10 minutes with oxygen and preincubated in stoppered tubes for 10 minutes at 37°C. Any additional compound was added halfway through this preincubation period. The reaction was initiated by adding the substrate, S-carboxymethyl-L-cysteine (5mg), in buffer (1ml at 37°C) and the mixture incubated in a stoppered tube for 30 minutes at 37°C in a shaking water bath. Aliquots (0.3ml) were removed at regular intervals during the first 5 minutes and then at 10, 20 and 30 minutes and added to 10% (w/v) trichloroacetic acid (0.2ml) which stopped the reaction. All samples were then frozen until analysis for sulphide and sulphoxide metabolites as previously described in detail (9). Results obtained were plotted graphically and the initial linear portion of the graph taken to calculate the sulphoxidation rates (nanomoles of sulphoxide formed / minute / milligramme of protein).

RESULTS AND DISCUSSION

Enzyme marker studies showed no cross contamination of the fractions, with lactate dehydrogenase activity being measurable only in the cytosol and cytochrome P-450 present only in the microsome fraction. The viability of the microsomes was confirmed by the formation of chlorpromazine sulphoxide from chlorpromazine, a known cytochrome P-450 mediated reaction (10).

The rates observed for sulphoxide formation in the incubates from various species are given in Table 1. In all species examined maximum activity was found in the cytosolic fraction with no detectable activity in the microsomes (< 0.05 nmoles / min / mg). The substrate itself was stable under the incubation conditions employed and did not spontaneously oxidise to form sulphoxides.

The addition of various compounds (c. 1mM) to incubates of rat, guinea pig and human liver cytosol showed that SKF525A, hydroxylamine, iproniazid, Cu^{+}, Mg^{2+}, Fe^{3+} and the addition of microsomes had no effect. Boiling the cytosolic fraction abolished activity and the addition of superoxide dismutase, EDTA (metal-chelator), and $HgNO_3$ (thiol inhibitor) all decreased activity. A 1.5-fold increase in activity was seen on the addition of Cu^{2+} ions and the presence of Fe^{2+} produced a 3.3-fold increase in activity in guinea pigs. The inclusion of ascorbic acid in the incubate also caused an increase in sulphoxide production but this was subsequently shown to be a chemical oxidation.

This study has shown that the sulphoxidation of a xenobiotic molecule, which is known to undergo

such a reaction in vivo (9,11), occurs preferentially within the cytosol and is not a substrate for microsomal oxidising systems. The structure of S-carboxymethyl-L-cysteine, a divalent alkyl sulphide, is similar to that of the naturally occurring amino acid cysteine and thus may be a substrate for the cysteine oxidase group of enzymes known to be components of the cytosol (and mitochondria) and which share some characteristics with the activity reported here, notably activation in the presence of Fe^{2+} ions (12). The properties of this cytosolic xenobiotic sulphoxidation system are now being further investigated.

Table 1 Rate of sulphoxide formation in cytosol from various species.

Species	Rate of sulphoxide formation (nmoles / min / mg)				
Rat[1]	F4	39.7 ± 12.1		M4	31.7 ± 8.4
Guinea pig[1]	F4	97.9 ± 6.3		M5	98.0 ± 8.8
Rabbit	F7	2.7 ± 1.8			
Hamster				M4	15.8 ± 5.1
Sheep	F3	6.3 ± 1.0			
Human[2]	F2	7.0	15.0	M1	7.0

Values are given as the mean ± one standard deviation except for the human livers where individual values are given.

[1] There was no statistically significant difference (Student's 't'-test) between male (M) and female (F) groups.

[2] The two human female values were taken from livers in the diseased state (alcoholic and cryptogenic cirrhosis).

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