THE BIOCHEMISTRY OF ENDOGENOUS ORGANOSULPHUR COMPOUNDS

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CONTENTS

SUMMARY

The role and metabolism of methionine and cysteine in mammals are described. An outline of the history of glutathione, of its function in maintaining the thiol status of the cell and of its protective action against oxidative stress, is given. The importance of glutathione reductase, glutathione peroxidases, glutathione-S-transferases in endogenous metabolism and of γ-glutamyltranspeptidase is described. The relationship between cysteine and glutathione is considered together with the inter-organ translocation of glutathione and its metabolism.

I. INTRODUCTION

Sulphate, the commonest compound of sulphur in the environment, when assimilated by plants is reduced and used for the synthesis of methionine and cysteine which can then be incorporated into protein. Mammals are unable to reduce sulphate and are ultimately dependent upon plant protein to provide their sulphur-containing amino acid requirement. In mammals, methionine and cysteine are metabolised by intricate pathways; the sulphur of unrequired amino acids is oxidised, then excreted as sulphate thus completing the sulphur cycle. The endogenous metabolism of methionine and cysteine has been reviewed /1,2/; this article aims to provide a brief discussion of the interrelationships of endogenous sulphur-containing compounds, and to place them in a biochemical content.

II. METABOLISM OF METHIONINE

Methionine, an essential amino acid, is required for protein synthesis and also forms part of the active centre of some enzymes. It is involved in combination with tRNA in the initiation of protein synthesis. When activated by reaction with ATP, methionine is converted to S-adenosyl-methionine (SAM) which functions as a methylating agent being itself converted to S-adenosylhomocysteine. Examples of methyl group acceptors are guanidoacetic acid, noradrenaline, phosphatidylethanol-amine, which are converted to creatine, adrenaline and phosphatidylcholine respectively; certain

lysine residues in proteins and some of the bases in t-RNAs also accept methyl groups from SAM. Homocysteine arising from S-adenosylhomocysteine is re-methylated at the expense of methyl-tetrahydro-folate and to a minor extent, in a reaction confined chiefly to the liver, using betaine. Homocysteine occupies a branch point in methionine metabolism /3/ for it may undergo irreversible transsulphuration; about 50% combines with serine to form cystathionine. Cystathionine is decomposed by cystathionase forming cysteine, NH_3 and α-ketobutyrate (Fig. 1). This enzyme is chiefly confined to the liver which therefore has a second source of cysteine in addition to that derived directly from the diet. Cysteine only becomes an essential amino acid if the supply of methionine is deficient or in the absence of cystathionase activity /4/.

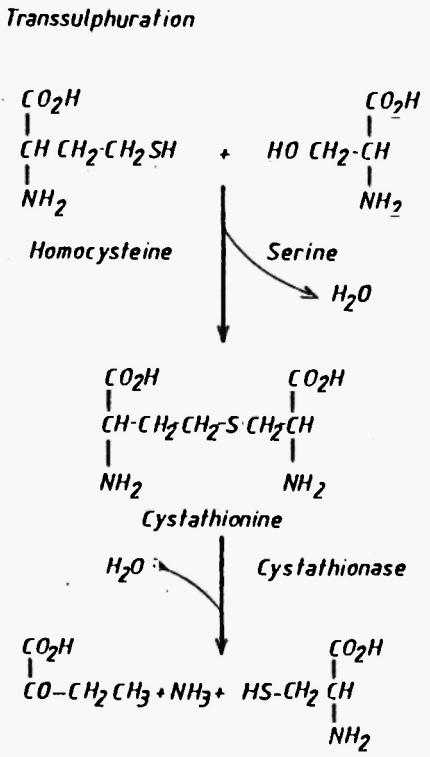

Fig. 1: Pathways of methionine metabolism. Figure illustrates the transsulphuration reaction and decomposition of cystathionine to cysteine.

In man the synthesis of polyamines may account for the metabolism of 2-5% of SAM. Transfer of $NH_2(CH_2)_3$- from decarboxylated SAM to putrescine yields spermidine and 5-methylthioadenosine, repetition of the reaction yielding spermine. Transamination of methionine to "ketomethionine" occurs only to a limited extent but subsequent decomposition of the keto acid leading to the formation of methanethiol may account for the toxic effects arising from the metabolism of large amounts of methionine. (Fig. 2)

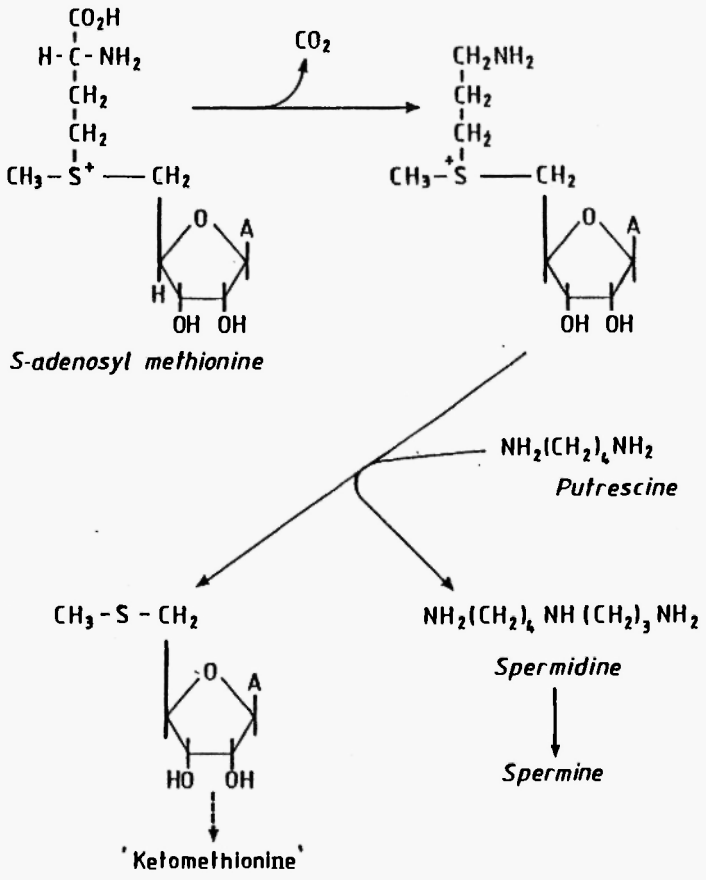

Fig. 2: Pathways of methionine metabolism. Figure illustrates role of S-Adenosyl-L-methionine in the synthesis of polyamines and transamination to ketomethionine.

III. METABOLISM OF CYSTEINE

The concentration of cysteine (30-200μM) /5/ in the tissues is lower than that of most other amino acids derived from protein. It is toxic in greater concentrations probably due to the high reactivity of the sulphydryl group; its reaction with pyridoxal phosphate may lead to a deficiency of that cofactor and it is easily oxidised to the relatively insoluble disulphide cystine which can accumulate to give rise to cystinosis /6/. The amino acid is obtained directly or via methionine from dietary protein. It is important in protein synthesis because cysteine residues brought into suitable positions by the conformation of the polypeptide chain may form disulphide bonds which then stabilise the conformation. Cysteine residues constitute part of the active centre of many enzymes. Cysteine is oxidised to cystine, the reaction being reversed by transhydrogenation with glutathione (GSH) and is required for the synthesis of GSH, taurine and coenzyme A.

The major metabolic pathway of cysteine involves oxidation to cysteinesulphinic acid, then transamination, via the unstable intermediate β-sulphinylpyruvate, to yield pyruvate and sulphite. This last is readily oxidised to sulphate. Some of this is activated by combination with ATP to give phosphoadenosinephosphosulphate (PAPS) the sulphating compound involved in synthesis of, for example, sulphate esters of carbohydrate residues in mucopolysaccharides or sulphate esters of steroids which are excreted in urine. The rest, however, is excreted as inorganic sulphate. Cysteinesulphinic acid is situated at a branch point in cysteine metabolism; decarboxylation yields hypotaurine, which is subsequently oxidised to the taurine used to form bile acid conjugates. There is marked species variation in the relative extents to which transamination and decarboxylation of cysteinesulphinic acid occur. An extreme is found in the cat which is deficient in the decarboxylase and therefore requires taurine as an essential amino acid /7/. Hypotaurine may also be formed by the oxidation of cysteamine arising from coenzyme A while dietary cysteic acid can be decarboxylated to yield taurine (Fig. 3).

Transamination of cysteine yields β-mercaptopyruvate which may be reduced to β-mercaptolactate or decarboxylated to thioacetate. Desulphhydration of cysteine is attributed to the action of cystathionase and yields NH_3, H_2S and pyruvate while a mitochondrial

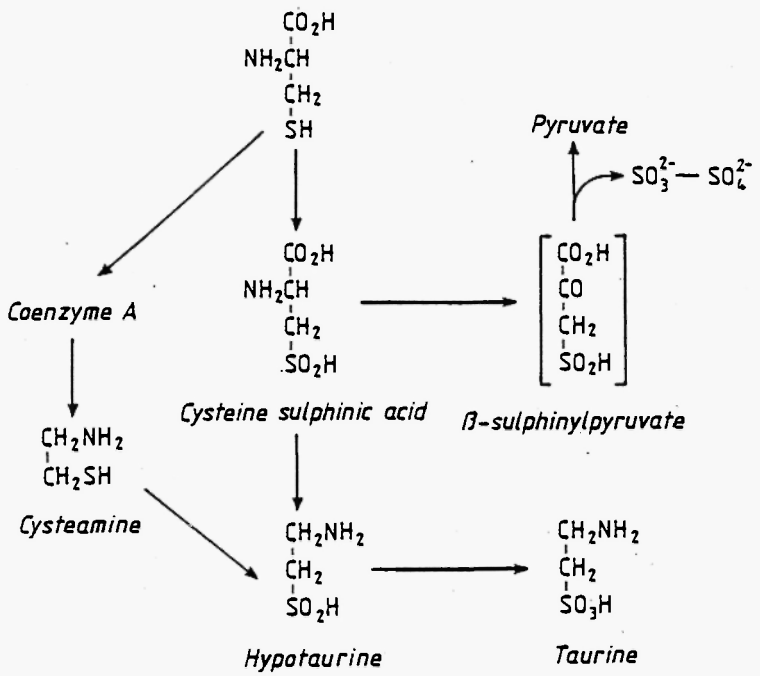

Fig. 3: Pathways of cysteine metabolism.

pathway leads to the formation of pyruvate, NH_3 and SO_4^{2-}. An enzyme has been described which catalyses the conversion of β-mercaptopyruvate to pyruvate and H_2S.

IV. HISTORY, METABOLISM AND FUNCTIONS OF GLUTATHIONE

4.1 Historical Aspects

The biological functions of the tripeptide, GSH, have been most intensively studied in the last forty years. It is widely distributed in microorganisms, plants and animal tissues and the diversity of its functions are such that research in the fields of enzymology, protein and nucleic acid synthesis, membrane structure, pharmacology and toxicology involves consideration of the role of the tripeptide. This has led to the publication of a large number of papers, several

reviews and books /e.g. 8-12/ discussing the many facets of the functions of GSH.

The history of GSH, recently described in detail /13/ begins in 1888 when de Rey-Pailhade /14/ prepared an aqueous extract of yeast which reduced sulphur to H_2S, a property later shown to be present in various animal tissue extracts. He postulated that the reducing property was due to a compound containing labile hydrogen, which he named philothion. Further reports led to the idea that the compound might contain the sulphydryl group and that the strong nitroprusside reaction given by protein-free extracts of tissues was due to cysteine, although this was not isolated /15/. The problem was investigated by Hopkins who reported /16/ in 1921 the isolation of a sulphur-containing reducing compound from yeast and muscle which on hydrolysis gave glutamic acid and cysteine. The compound believed on this evidence to be a dipeptide was thought to be identical with philothion and was re-named glutathione. A few years later a preparation of glutathione was obtained which appeared to contain a third amino acid /17/. The isolation and properties of GSH were re-investigated by Hopkins /18/ and the third amino acid was identified as glycine. The structure of the tripeptide was elucidated from its chemical reactions /19/ and was finally established as γ-glutamylcysteinylglycine by synthesis carried out by Harrington and Mead /20/.

4.2 Thiol reactivity

The importance of GSH and its oxidised form GSSG in maintaining the thiol status of the cell was early recognised. GSSG is reduced by glutathione reductase at the expense of NADPH, the ratio GSH/GSSH in the cell being normally 20:1. Cystine is also reduced by transhydrogenation with GSH as are the disulphide bonds of insulin and other proteins. The latter reaction was catalysed by GSH:insulin transhydrogenase, an enzyme found to have broad substrate specificity /21/. The formation of disulphide bonds in nascent proteins involves GSSG and an enzyme, protein disulphide-isomerase. A further enzyme, thiol-protein-disulphide oxidoreductase was isolated from rat liver. The similarity of the physicochemical properties of the three enzymes described, which are active in the synthesis and scission of protein disulphide bonds, suggests that these enzymes are closely related /22/. The redox couple GSH/GSSH

may be important in regulating enzyme activity; for example in glycogen synthetase D /23/

$$\underset{\text{(active)}}{\text{Glycogen synthetase D-SH}} + \text{GSSG} \rightleftarrows \underset{\text{(inactive)}}{\text{Glycogen synthetase D-SSG}} + \text{GSH}$$

Similary, the low activity of a carbonic anhydrase isolated from erythrocytes of the tiger shark has been attributed to the existence of approximately 3 mol-cysteine and 3 mol GSH bound in disulphide linkage per mol. of enzyme /24/.

In addition to its importance in protein biochemistry, GSH/GSSG is involved in the fundamental reduction of ribonucleotides to deoxyribonucleotides required for DNA synthesis.

The structural integrity of the cell also depends upon the level of GSH. Incubation of isolated hepatocytes in a medium containing bromobenzene resulted in a depletion of the cellular GSH with a concomitant decrease in extra-mitochondrial Ca^{2+}. The consequent harmful effect on the hepatocyte cytoskeleton was evidenced by increased blebbing of the plasma membrane /25/, demonstrating the requirement for GSH to retain integrity.

4.3. Mercapturic acid synthesis and GSH transferases

Considerable interest in GSH arose from its possible involvement in the synthesis of the mercapturic acids which were identified as metabolites of monohalogenobenzenes even before the history of GSH began /26, 27/. These metabolites were found to be derivatives of N-acetylcysteine. Over the years several different types of mercapturic acid precursors were recognised /28/ and attention turned to the mechanism of mercapturic acid formation and particularly to the immediate source of cysteine required for their synthesis. Waelsch /29/ pointed out as early as 1930 that GSH contained the three amino acids used in the detoxication of foreign compounds. While it was shown /30/ that GSH was not the immediate source of glycine for hippuric acid synthesis, evidence accumulated that it was the immediate source of cysteine for mercapturic acid formation. There were two lines of evidence; firstly the administration of mercapturic acid precursors to animals resulted in a fall in tissue GSH levels, particularly in the liver /28/; secondly the results of Boyland and his coworkers showed that

several enzymes catalysed the conjugation of different mercapturic acid precursors but all the enzymes were specific for the second substrate, GSH /31/. These enzymes, the GSH-S-transferases, have been extensively investigated /32, 33/ and are now regarded as a family of enzymes which with GSH form a defensive system against xenobiotics comparable with the cytochrome P450 system. It is also recognised that the glutathione-S-transferases are involved in certain endogenous metabolic processes.

GSH and GSH transferases are involved in the synthesis of leukotriene C, a GSH-conjugate, from the epoxide leukotriene A, /34/ and also catalyse steroid isomerisation /35/ and the synthesis of prostaglandin D from prostaglandin endoperoxides /36/. Other enzymes using GSH are formaldehyde dehydrogenase and glyoxalase which detoxifies endogenous α-oxoaldehydes while γ-glutamyl-transpeptidase catalyses the first step in the degradation of GSH. The mechanism of action of GSH-dependent enzymes has been reviewed /37/.

GSH exerts a protective action against endogenous H_2O_2; this continually leaks from the mitochondrial and microsomal cytochrome systems and can also be generated by superoxide dismutase from superoxide anions arising in these systems /38/. Glutathione peroxidase, a Se-containing enzyme, is as effective as catalase at promoting the decomposition of H_2O_2 at physiological concentrations /39/. Lipid hydroperoxides also serve as substrates

$$H_2O_2 + 2\,GSH \longrightarrow 2H_2O + GSSG$$
$$ROOH + 2\,GSH \longrightarrow ROH + H_2O + GSSG$$

A second glutathione peroxidase (not Se-dependent) acts upon lipid hydroperoxides but does not reduce H_2O_2 Several glutathione S-transferases possess GSH-peroxidase activity for hydroperoxides; these isoenzymes contain subunits 1,2,3 and 7 /32/.

A previously uncharacterised iso-enzyme from rat foetal liver has been reported which possesses GSH-S-transferase activity towards several substrates together with a high GSH-peroxidase activity /40/. This may protect the foetus against the possible teratogenic effects of organic peroxides.

Generally, the action of glutathione peroxidases combined with GSSG reductase protects the cell from the harmful effects of free radicals which may increase in oxidative stress.

4.4. Synthesis and degradation of glutathione

In contrast to the level of cysteine occurring in the tissues (30-200μM) the concentration of GSH is in the range 0.5-10 mM. In erythrocytes the concentration is about 1 mM while in plasma it is about 5μM; little is normally present in saliva or urine. The glutathione content of liver in the rat is subject to a circadian rhythm, peak concentration corresponding to the feeding period /41,42/.

TABLE 1

Levels of GSH and GSSG in Tissues

	GSH mM	GSSG mM	½ life (h)
Liver	4.5-6.5	0.06-0.13	2-4
Kidney	2.5	0.06	0.5
Intestine	3.5		
Lung	1.5		
Brain	1.0	0.04	
Eye lens	2.6-12.0		72
Whole blood	0.93-1.2	0.02	
Erythrocyte	2-3	0.005-0.13	65-69
Plasma	.005-.025		
Saliva	.002		
Urine	.001		

Hepatic GSH concentration is not uniform; higher amounts occur in the periportal region decreasing towards the centrilobular region /43/. Two distinct pools of total glutathione are thought to exist in the hepatocyte, 87.2% being in the cytosol and 12.8% in the mitochondrial matrix /44/.

Synthesis of GSH is effected by γ-glutamylcysteine synthetase (i) and glutathione synthetase (ii).

Glutamic acid + cysteine + ATP —> γ-glutamylcysteine + ADP + Pi (i)

γ-Glutamylcysteine + glycine + ATP —> γ-glutamylcysteinylglycine(GSH) + ADP + Pi (ii)

GSH competitively inhibits γ-glutamylcysteine synthetase; the availability of cysteine is the limiting factor in the synthesis as clearly demonstrated in isolated hepatic and renal cells /45/.

The γ-glutamylcysteine link in GSH is not susceptible to the action of peptidases and the first step in the degradation of GSH is brought about by γ-glutamyltranspeptidase (γ-GT). The glutamyl residue is transferred to an amino acid, or peptide (cystine being a highly suitable acceptor) or to H_2O. The cysteinylglycine is hydrolysed by cysteinylglycine peptidase. The distribution of γ-GT is of particular interest; it is located in the outer cell membrane, regions of intense activity occurring in many tissues. Relative activities to that in the kidney (100) are pancreas, (20), jejeunal villi (3.3). liver (0.2). The low hepatic activity is largely confined to the bile canaliculi.

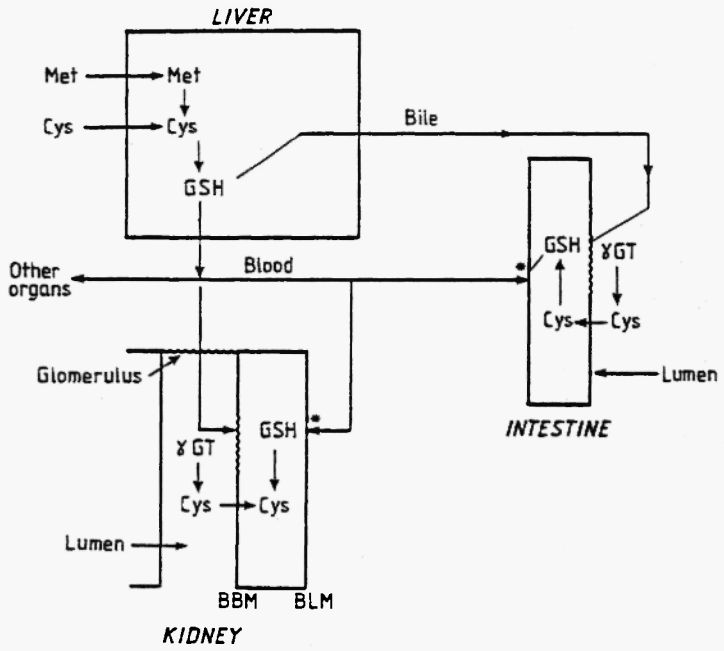

Fig. 4: Synthesis and degradation of glutathione. BBM = brush border membrane; BLM = basolateral membrane; * = Na transport system; γ-GT = γ-GT activity.

The γ-glutamyl cycle for the absorption of γ-glutamyl–amino acids was proposed /46/. Briefly intracellular GSH is translocated to the outer cell membrane where it is acted on by γ-GT to give a γ-glutamyl amino acid (Glu AA) which passes into the cell together with cysteinylglycine or the products of its hydrolysis. GluAA is decomposed by γ-glutamylcyclotransferase to give 5-oxoproline and the amino acid; 5-oxoprolinase converts 5-oxoproline to glutamic acid which becomes available for GSH synthesis with cysteine and glycine from the dipeptide. Evidence for the role of this cycle comes from the location of γ-GT in regions where rapid absorption of amino acids occurs and also from the increased activity of γ-GT in rat mammary gland as lactation is established, thus providing for the high demand for amino acids in the synthesis of mammary tissue and milk protein /47/. Deficiency of γ-GT leads to excretion of excess GSH; mice injected with a selective inhibitor of γ-GT excreted about 800 times the normal amount of urinary GSH in 1 h after the dose, while glutathionuria was seen in a patient with an inborn γ-GT deficiency /48/.

It was shown /49/ that 2 h after mice were dosed with the selective γ-glutamylcysteine synthetase inhibitor, buthionine sulphoximine, the levels of GSH fell to 18% of the control value in kidney, 35% in liver, 46% in pancreas, 67% in skeletal muscle with smaller falls to 82-93% of the control values in brain, heart, lung and intestinal mucosa. Thus the rate of GSH synthesis is highest in kidney followed by liver and pancreas; in other tissues the rate is much less.

Cysteine is maintained in the reduced form in the cell but is oxidised in plasma, the predominant form being cystine /5/. It is likely that GSH, present in liver at a concentration ten times that of cysteine, functions as a reservoir of cysteine and as a vehicle for its transport /50/. Hepatocytes are permeable to cysteine and methionine but not to cystine; however, the high hepatic cystathionase activity ensures that a supply of cysteine from methionine is available so that the liver is well equipped to synthesise and export GSH; the tripeptide is not decomposed as it reaches the outer hepatocyte membrane from which γ-GT is absent. GSSG is excreted via the bile by a mechanism shared by GSH-conjugates /51/ to the intestine where there is considerable γ-GT activity. Plasma GSH concentration is about 5 μM and its turnover is rapid; at least 80% is removed by the kidney in a single pass. The fraction which is filtered through the

glomeruli encounters the γ-GT of the nephron brush border and is absorbed as the constituent amino acids. Plasma GSH is also absorbed through the basal-lateral membrane on which some γ-GT is situated but in which a Na-dependent GSH transport system is also present /52, 53/ (Fig. 4). Although kidney cells take up methionine the kidney is deficient in cystathionase and cannot use methionine as a source of cysteine. In contrast to hepatocytes, renal cells readily take up cystine, which can provide an alternative source of cysteine.

Glutathione thus has a wide range of biological functions, unrivalled by any other small organic molecule. All, however, involve in some way the maintenance of homeostasis.

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