

The Importance and Regulation of Hepatic Glutathione

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Glutathione plays a key role in the liver in detoxification reactions and in regulating the thiol-disulfide status of the cell. Glutathione synthesis is regulated mainly by the availability of precursor cysteine and the concentration of glutathione itself which feeds back to regulate its own synthesis. Degradation of hepatic glutathione is principally regulated by the efflux of reduced and oxidized glutathione into both sinusoidal plasma and bile. In addition, glutathione may be consumed in conjugation reactions. Under conditions of oxidative stress, the liver exports oxidized glutathione into bile in a concentrative fashion, whereas under basal conditions, mainly reduced glutathione is exported into bile and blood. The mechanism of export of reduced glutathione into bile and sinusoidal blood is poorly understood.

Glutathione is a tripeptide, γ -glutamylcysteinylglycine, which appears to have key functions in protective processes (Table 1). The subject of the chemistry and functions of glutathione are extensively reviewed by Kosower and Kosower [1]. Briefly, glutathione participates in detoxification reactions of electrophilic substances, such as carcinogen-epoxide metabolites and certain drugs by conjugation between nucleophilic thiol of glutathione and an electrophilic site on another molecule. Hydrogen peroxide and lipid peroxides are also detoxified by reduced glutathione, generating oxidized glutathione. Both types of reactions can occur chemically and enzymatically. The glutathione S-transferases are responsible for the former reactions and have been extensively discussed recently [2]. The glutathione peroxidases (selenium-dependent and independent) are responsible for the enzymatic reduction of peroxides [3]. Thus, glutathione participates in important detoxification reactions. In addition, the ratio of reduced to oxidized glutathione is critical in regulating protein and enzyme functions and protein synthesis, and the maintenance of the redox state [1]. Excess oxidized (as would occur with reduction of peroxides or with a change in the redox potential) leads to the interaction of oxidized glutathione with protein-SH groups, forming mixed disulfides [4] (Fig. 1). Conversely, maintaining the normal concentration of reduced glutathione supports the maintenance of the reduced state of protein thiols. Formation of glutathione-protein mixed disulfides has been shown to alter a variety of cell functions, including enzyme function, protein synthesis, cell integrity, microtubular function, transport processes, and release mechanisms [1,5]. Since oxidized glutathione, when present in

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TABLE 1
Functions of Glutathione

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1. Detoxification of electrophiles: Glutathione S-transferases
 2. Reduction of peroxides and radicals: Glutathione peroxidases (Selenium-dependent and independent)
 3. GSH/GSSG ratio
 - a. Maintenance of redox state
 - b. Protein synthesis
 - c. Microtubular function
 - d. Adenylate cyclase regulation
 - e. Transport and release of neurotransmitters
-

excess, can exert noxious effects on cell function, mechanisms for handling this substance are extremely critical. Because of the importance of both reduced and oxidized glutathione, glutathione homeostasis is considered in more detail below.

GLUTATHIONE SYNTHESIS

Glutathione is synthesized directly from precursor amino acids. Two cytosolic enzymes are involved, both of which require ATP and Mg^{++} or Mn^{++} . The first, γ -glutamylcysteine synthetase, is rate-limiting, and the second, glutathione synthetase, adds glycine. The product, glutathione, regulates its own synthesis by non-allosteric competitive inhibition of the γ -glutamate binding site of the first enzyme [6,7]. The K_i for glutathione and the K_m for glutamate are approximately 2 mM; the physiologic concentration of glutamate in liver is approximately 2 mM whereas the concentration of glutathione in liver is approximately 7 mM [8]. Nearly 90 percent of hepatic glutathione is found in cytosol. Our studies of specific binding of glutathione in cytosol indicate a single class of binding sites ($K_d = 0.1$ mM) with a capacity of 0.2 mmol/g liver (unpublished observations). Glutathione binding in cytosol is accounted for mainly by the glutathione S-transferases, which have a $K_m = 0.1$ mM for glutathione and account for 10 percent of cytosol protein [2]. Only a small fraction of cytosol glutathione could be accommodated by binding to these enzymes. Therefore, it can be concluded that the bulk of cellular glutathione exists unbound.

The availability of the precursor amino acid, cysteine, is of critical importance in glutathione synthesis. The physiologic concentration of cysteine is about one order of magnitude lower than the K_m of γ -glutamylcysteine synthetase for cysteine [7].

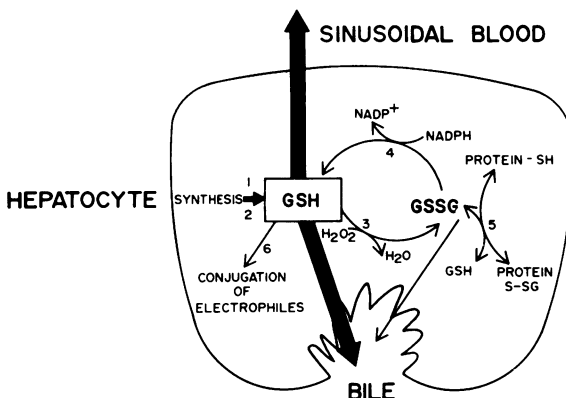


FIG. 1. The normal glutathione status. **GSH**, reduced glutathione; **GSSG**, oxidized glutathione; 1, γ -glutamylcysteine synthetase; 2, glutathione synthetase; 3, glutathione peroxidase; 4, glutathione reductase; 5, thiol disulfide transferases; 6, glutathione S-transferases.

Moreover, the source of cysteine in hepatocytes appears to be principally methionine [9-11]. Cysteine is poorly taken up by hepatocytes whereas methionine is readily taken up and metabolized to S-adenosylmethionine, homocysteine, cystathione, and cysteine in sequence [11,12]. The kidney differs in that cysteine, as cystine, is readily taken up by tubular epithelium, supporting the requirements for the rapid turnover of glutathione in kidney [12].

THE FATE OF OXIDIZED GLUTATHIONE

Within cells, oxidized glutathione can undergo reactions with tissue thiols to form mixed disulfides perhaps catalyzed by thiol-disulfide transferases [4]. Substantial amounts of glutathione seem to be "stored" in this way and the normal diurnal variation in reduced/oxidized glutathione seems to be closely associated with this phenomenon [4]. In addition, oxidized glutathione can be enzymatically reduced by glutathione reductase and NADPH [1]. Oxidized glutathione (GSSG) usually exists in very small concentrations in cells (5 percent of total glutathione equivalents in liver). The maintenance of low GSSG concentrations is accounted for by its reacting very rapidly with protein thiols, its reduction by NADPH glutathione reductase, and its active transport out of the cells. In liver the latter seems to occur preferentially into bile. It is remarkable that under conditions of oxidative stress associated with increased formation of GSSG, the concentration of GSSG does not increase significantly in liver. Thus, the mechanisms cited for handling GSSG insure that it does not accumulate. Rapid efflux (export) appears to be of critical importance in ridding cells of GSSG and avoiding its potential noxious effects.

ENZYMATIC DEGRADATION OF GLUTATHIONE

Glutathione may be consumed by conjugation reactions (glutathione S-transferases) which mainly involve metabolism of xenobiotic agents [2]. However, the major enzymatic degradation normally involves the action of γ -glutamyltranspeptidase [13,14], a brush border enzyme of renal tubular cells [15], and intestinal epithelium [16] for which no significant role has been demonstrated in liver [17]. The principal mechanism of hepatocyte glutathione turnover appears to be efflux [3,18]. This contention is based on comparison of the rate of efflux of hepatic glutathione into the perfusate of isolated perfused liver compared to published estimates of hepatic glutathione half-life. However, no direct comparison of quantitative efflux versus steady-state hepatic glutathione turnover has been made.

EFFLUX OF GLUTATHIONE

In lymphoid lines with high, medium, and low membrane γ -glutamyltranspeptidase, the efflux of glutathione occurs independently of this enzyme activity [19]. Apparently all cells efflux reduced glutathione, and those cells with significant surface γ -glutamyltranspeptidase degrade the extracellular glutathione by oxidation and hydrolysis. Reduced and oxidized glutathione in extracellular perfusate are not taken up or metabolized by the perfused liver [20]. However, when injected intravenously both rapidly disappear, about two-thirds going to the kidney where these peptides are ultrafiltered and are degraded by γ -glutamyltranspeptidase in the tubular lumen brush-border. The component amino acids either redistribute to the liver and other organs or are used to maintain intrarenal glutathione [20,21].

The fate of biliary glutathione is unknown. Isolated intestinal cells metabolize extracellular glutathione by γ -glutamyltranspeptidase reaction [22], but this has not been studied in the intact organ. However, the existence of a similar process as ob-

served in the kidney involving γ -glutamyltranspeptidase [16,23] in the intestinal brush-border seems tenable. It is possible that the efflux of liver glutathione into bile and blood not only is a major control mechanism for hepatic glutathione homeostasis, but is also important for supplying other organs rich in surface membrane γ -glutamyltranspeptidase (e.g., kidney and intestine) with the constituent amino acids necessary for glutathione synthesis.

Numerous studies have evaluated the efflux of glutathione from the liver into perfusate of the isolated perfused liver and the suspending medium of isolated hepatocytes. The mechanism of efflux into perfusate (or plasma) is poorly understood. In liver perfusion studies, perfusate glutathione is nearly complete in the reduced form [19]. *In vivo*, higher concentrations of reduced glutathione (20–30 μ M) are found in hepatic vein plasma of the rat compared to arterial and systemic venous levels (5–10 μ M), reflecting the efflux of reduced glutathione from the liver into the plasma [24].

Preliminary work by others and confirmed in my laboratory points to biliary glutathione efflux from liver [25,26]. According to a preliminary study in the perfused liver, bile efflux was exclusively in the form of oxidized glutathione, and represented less than one-half the output of glutathione equivalents into perfusate [25]. We have found that reduced glutathione rapidly oxidizes in bile *in vitro* ($t_{1/2} = 5$ minutes) [27], and that when collected under proper conditions, the bulk (80 percent) of glutathione equivalents excreted in bile are in the reduced form [26,27]. During oxidative stress, such as lipid peroxidation, the increased formation of oxidized glutathione in liver results in preferential increased efflux of the latter into bile [3]. As oxidized glutathione formation is further enhanced it then begins to be exported across the sinusoidal surface into perfusate [25]. Bile contains oxidized glutathione at a higher concentration than liver suggesting an active transport process. Active transport (export) of oxidized glutathione has been proposed in erythrocytes [28]. Recently Kondo et al. demonstrated that inside-out vesicles from human erythrocytes actively transported oxidized glutathione but not reduced glutathione [29].

GLUTATHIONE TURNOVER

Glutathione turnover studies have been performed using incorporation of various precursors. Using N^{15} glutamate, ^{14}C -glycine, ^{35}S -cysteine, and ^{35}S -glutathione, a broad spectrum of hepatic glutathione turnover rates have been described in various species with $t_{1/2}$ ranging from two hours to greater than eight hours [30–36]. Tateishi et al. [35] have described an accelerated turnover with fasting (mechanism unknown) and suggest that there are two pools of hepatic glutathione with $t_{1/2}$ 1.7 and 28.5 hours. However, Lauterburg et al. recently suggested that the longer half-life seen by Tateishi corresponds to protein degradation and that the turnover studies support a single pool of hepatic glutathione. Lauterburg et al. [36] demonstrate several other important points in their studies: (1) there is an inverse relationship between turnover and age (weight); (2) depletion of glutathione with diethylmaleate increased turnover and synthesis, presumably because of decreased feedback (nonallosteric) inhibition of glutathione synthesis following glutathione depletion; (3) turnover rate is the same when studied with radioactive cysteine, glutamate, glycine, and glutathione. They demonstrated that even in fed animals the turnover of precursors is very rapid relative to that of glutathione and therefore would not be expected to influence the time course of specific radioactivity of glutathione.

INDUCTION OF HEPATIC GLUTATHIONE

Treatment of animals with a number of xenobiotics has been shown to raise he-

patic glutathione levels [37–40]. This is referred to as induction, although in no case has the influence of xenobiotics on glutathione turnover (synthesis and degradation) been evaluated. We have studied the effect of phenobarbital, which increases hepatic glutathione concentration by 20–30 percent. Phenobarbital has no effect on the enzymes involved in glutathione synthesis or the concentration of precursor substrates (precursor amino acids) [37]. Similar results have been found with 3-methylcholanthrene (unpublished observations). Thus, two major classes of inducing agents for microsomal mixed function oxidases have a modest inducing effect on hepatic glutathione by unknown mechanisms. The induction of specific intracellular binding sites for glutathione, such as to the glutathione S-transferases, can only account for a very small fraction of the increase in glutathione concentration in response to these xenobiotics. The mechanism for this apparent induction is not understood.

In summary, glutathione is a vital substance in detoxification and cell physiology. Its regulation in liver is based on a homeostatic feedback inhibition mechanism. The availability of cysteine is a critical factor in the regulation of synthesis. Turnover in liver is determined mainly by the efflux of glutathione into both sinusoidal blood and bile and its subsequent degradation. The constituents of exported glutathione are conserved by hydrolysis and cellular uptake mainly in the kidney and intestine as governed by brush-border γ -glutamyltranspeptidase. Thus, one can view the liver as a glutathione-generating factor which supplies the kidney and intestines with the constituents for glutathione resynthesis.

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