Biosynthesis and Functions of Glutathione, an Essential Biofactor

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I. INTRODUCTION

Glutathione (L-γ-glutamyl-L-cysteinyl-glycine) is a biofactor that is present in almost all living cells. It is usually found in rather high concentrations as compared, for example, to cysteine and other amino acids. Although glutathione is an essential biofactor, it is not a vitamin. Thus, it is not required in the diet of animals although it is present in many of the plant and animal constituents of diets. Glutathione is synthesized within cells from glutamate, cysteine, and glycine. Glutathione provides cells with their reducing environment and protects by reacting directly with reactive oxygen compounds, and less directly by maintaining in reduced forms other compounds including cysteine, protein thiols, ascorbate and α-tocopherol. The cellular requirement for glutathione may have evolved as a protective agent against oxygen and has been adapted through evolution to perform a variety of metabolic, protective, and catalytic functions. These functions are indicated in Fig. 1. [For recent reviews of the literature, see (1-4)].

Outline of the biochemistry of GSH. AA, amino acids; X, compounds that react with GSH to form conjugates. 1, γ-glutamylcysteine synthetase; 2, GSH synthetase; 3, γ-glutamyltranspeptidase; 4, dipeptidases; 5, γ-glutamylcycloleptidase; 6, 5-oxoprolinase; 7, GSH S-transferases; 8, N-acetyltransferase; 9, GSH peroxidases; (Se-containing and non Se-containing); 10, GSH thiol transferases such as glutaredoxin and protein disulfide isomerase; 11, reaction of free radicals with GSH; 12, glutathione disulfide (GSSG) reductase; 13, transport of γ-Glu-(Cys)2; 14, GSH functions as a coenzyme for formaldehyde dehydrogenase, maleylactacetate isomerase, glyoxalase, prostaglandin endoperoxidase isomerasers, and dichlorodiphenyltrichloroethane (DDT)-dehydrochlorinase and similar enzymes. In the glyoxalase reaction, the hemimercaptal formed nonenzymatically by reaction of methylglyoxal and GSH is converted by glyoxalase I to S-lactyl-GSH, which is split by glyoxalase II to D-lactate and GSH. In the formaldehyde dehydrogenase reaction, S-formyl GSH is formed (GSH + HCHO + NAD+) and hydrolyzed to formate and GSH (Modified from (4)).
Synthesis of Glutathione and Its Inhibition

The reactions of the \( \gamma \)-glutamyl cycle account for the synthesis of glutathione, which occurs in two steps catalyzed by \( \gamma \)-glutamylcysteine synthetase and glutathione synthetase. The first step in

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(1) \quad L-\text{Glutamate} + L-\text{cysteine} + \text{ATP} \rightarrow L-\gamma-\text{glutamyl-L-cysteine} + \text{ADP} + P_i \\
\text{(Catalyzed by \( \gamma \)-glutamylcysteine synthetase)}
\]

\[
(2) \quad L-\gamma-\text{Glutamyl-L-cysteine} + \text{Glycine} + \text{ATP} \rightarrow \text{Glutathione} + \text{ADP} + P_i \\
\text{(Catalyzed by glutathione synthetase)}
\]

glutathione biosynthesis is feedback inhibited by glutathione. This reaction may be selectively inhibited by certain amino acid sulfoximines, which bind at the glutamate site of \( \gamma \)-glutamylcysteine synthetase and are phosphorylated on the sulfoxime nitrogen atoms to yield the corresponding amino acid sulfoximine N-phosphates (5-7). These bind tightly but non-covalently to the enzyme thus inhibiting it irreversibly. Buthionine sulfoximine (S-n-butylhomocysteine sulfoximine) has been widely used in vitro and in vivo for inhibition of glutathione biosynthesis.

Modulation of Glutathione Metabolism: Tumor Therapy

Studies on the synthesis of glutathione and its utilization have elucidated the biochemistry and functions of this biofactor and have led to effective methods for decreasing and for also increasing cellular glutathione (8). Modulation of cellular glutathione has provided a new approach to the treatment of certain tumors, especially cell types that are resistant to drugs and to radiation. Thus, decreasing the synthesis of glutathione, which can be achieved by giving an inhibitor of \( \gamma \)-glutamylcysteine synthetase, sensitizes certain tumors to radiation and to the effects of chemotherapeutic agents. Certain tumors that are resistant to a variety of drugs and to radiation appear to require glutathione for such resistance, which may be reversed by decreasing the levels of glutathione in the tumor and by decreasing the capacity of the tumor to synthesize glutathione. It was initially assumed that the level of glutathione in resistant tumors (which is often 1.5-2 times higher than found in the corresponding sensitive tumors) is the major determinant of resistance. This may be true for some tumors (and anticancer agents), but there is now evidence that the capacity of the tumor to synthesize glutathione is also of importance; in some tumors, the capacity to synthesize glutathione may be more important than the level of glutathione (9,10). The treatment of drug-resistant and radiation-resistant tumors by administration of buthionine sulfoximine constitutes a rational approach to therapy; clinical trials with buthionine sulfoximine are now in progress.

Increase of Cellular Glutathione

Another therapeutically useful modulation of glutathione metabolism involves procedures that increase the glutathione levels of cells. For example, normal cells may be selectively protected against toxic compounds, including antitumor agents, and against the effects of radiation, by administration of cysteine-delivery and glutathione-delivery agents. Although administration of cysteine may increase glutathione levels, this amino acid is toxic and therefore alternative ways of delivering cysteine to the cell have been considered (11). A cysteine derivative in which the thiol group is masked and which is converted intracellularly to cysteine is L-2-oxothiazolidine-4-carboxylate (12). This compound is a substrate of the enzyme 5-oxoprolinase, which converts 5-oxoprolprine to glutamate in the normal ATP-dependent
reaction. After administration of L-2-oxothiazolidine-4-carboxylate, (which is non-toxic and readily transported), to mice and rats, tissue levels of cysteine and glutathione increase substantially (13,14). Interestingly, many tumors do not have significant levels of 5-oxoprolinase and therefore not active in conversion of L-2-oxothiazolidine-4-carboxylate to L-cysteine. Thus, administration of L-2-oxothiazolidine-4-carboxylate would not increase glutathione levels in such tumors, but would increase glutathione levels in many normal tissues, thus increasing the resistance of normal cells to the toxic effects of a drug without producing a corresponding increase in the resistance of the tumor cells.

Another way of increasing cellular glutathione is to administer glutathione esters. In contrast to glutathione, glutathione esters are readily transported into cells and are hydrolyzed into glutathione intracellularly. The effectiveness of monoester of glutathione (in which the glycine carboxyl group is esterified) in increasing cellular glutathione levels has been extensively documented (15-27). Although administration of glutathione may lead to small increases in intracellular glutathione, such increase is not the result of significant transport of intact glutathione itself, but rather to its extracellular degradation followed by uptake of the products and intracellular synthesis of glutathione. That this mechanism is operative may be demonstrated by application of appropriate concentrations of inhibitors of glutathione synthesis or of γ-glutamyl transpeptidase. The effective transport of glutathione esters underlies their use in protecting cells against acetaminophen (15), heavy metal ions (20,21), and certain anticancer agents (e.g., melphelan, cisplatin, cyclophosphamide (19,22)). Glutathione monoester administration also protects against cellular damage associated with administration of buthionine sulfoximine as discussed below.

Glutathione Deficiency and Its Reversal

An experimental model has been used in which the synthesis of glutathione is selectively blocked by administration of buthionine sulfoximine. That glutathione deficiency produced by inhibition of its synthesis cannot be prevented or reversed by giving glutathione supports the conclusion that there is very little transport of intact glutathione into cells. Such glutathione deficiency can however, be prevented or reversed by administration of glutathione esters. Thus, in this model, glutathione deficiency is produced, in the absence of applied stress, by inhibition of γ-glutamylcysteine synthetase, and it is prevented or reversed by administration of glutathione esters; this approach has been used in adult mice and in newborn mice and rats. Newborns treated with buthionine sulfoximine in relatively small doses develop cataracts which may be prevented by administration of glutathione esters (23). In adult mice, glutathione deficiency leads to cellular damage in muscle (24), lung (25), lymphocytes (25), jejunum (26), and colon (26). In newborn rats, there is damage also to liver, kidney, and brain (27-29). In all instances, cellular damage, which may be prevented by administration of glutathione esters, is associated with mitochondrial degeneration. It is notable that a significant fraction of the oxygen utilized by mitochondria is normally converted to hydrogen peroxide (30-33). Mitochondria lack catalase and thus depend upon the action of glutathione peroxidases for disposal of the hydrogen peroxide formed. Mitochondria do not have the enzymes required for glutathione synthesis, but transport it from the cytosol (34,35). Studies on isolated rat liver mitochondria have provided evidence for at least two glutathione transporters with apparent Km values of 60 μM and 5 mM, respectively (35). When glutathione levels are greatly decreased, mitochondria undergo substantial swelling and degeneration and are eventually completely destroyed often resulting in severe vacuolization (visualized by electron microscopy).

Relationships Between Glutathione and Ascorbate

It has long been thought that the reduction of dehydroascorbate to ascorbate involves reaction with glutathione (36-39), and recent studies indicate that this reaction is catalyzed by glutaredoxin and protein disulfide isomerase (40). Treatment of newborn rats with buthionine...
sulfoximine leads to markedly decreased tissue levels of both ascorbate and total ascorbate (dehydroascorbate plus ascorbate) indicating that there is formation and breakdown of dehydroascorbate (28). Mitochondrial and total tissue levels of glutathione were greatly decreased after treatment with buthionine sulfoximine; when ascorbate was given together with buthionine sulfoximine the levels of glutathione were significantly higher than when buthionine sulfoximine was given alone, indicating a sparing effect of ascorbate on glutathione. Newborn rats treated with buthionine sulfoximine at a daily dose of 6 mmol/kg exhibit high mortality and die within 4-6 days. Treatment with buthionine sulfoximine plus ascorbate (2 mmol/kg/day) led to marked reduction in mortality. Dehydroascorbate was not effective in preventing mortality and smaller doses of ascorbate were less effective or not effective. A similar result was obtained in studies in which newborn rats were given only 2 doses of buthionine sulfoximine (3 mmol/kg at 36 and 60 hours of age). Such animals are found to have bilateral cataracts when they open their eyes on the 14th or 15th day of life. Such cataract formation is prevented by administration of glutathione monoethyl ester (23) and also by giving ascorbate (28).

These and related findings (29) indicate that glutathione and ascorbate have similar antioxidant actions. The data indicate that an important physiological function of glutathione is to maintain ascorbate in reduced form. Although it appears that glutathione functions normally to maintain ascorbate and probably α-tocopherol (and other cellular constituents) in their reduced forms, it is of interest that ascorbate can serve as an essential antioxidant when there is a severe deficiency of glutathione. Ascorbate may function, under physiological conditions, in reactions in which glutathione does not efficiently participate, and vice versa. It seems interesting that glutathione and ascorbate can replace each other under certain conditions. The linkage between glutathione and ascorbate appears to be of physiological significance.

It is interesting to note that guinea pigs are highly sensitive to depletion of glutathione and, like newborn rats, die soon after receiving several doses of buthionine sulfoximine (41,42). Induction of ascorbate synthesis, which occurs transiently in adult mice as a consequence of glutathione deficiency, does not occur in newborn rats, nor does it occur in guinea pigs (42,43), which, like humans, do not synthesize ascorbate.

Model for Oxidative Stress

Inhibition of glutathione synthesis in the newborn rat provides a potentially useful model of oxidative stress in which the normal endogenous physiological formation of reactive oxygen species is largely unopposed (29). Oxidative tissue damage occurs in association with decreased levels of ascorbate and mitochondrial degeneration. The lung contains decreased number of lamellar bodies and there is a decrease of intraalveolar surfactant. There is proximal renal tubular damage as well as focal necrosis in the liver with evidence of decreased extramedullary hematopoiesis. Marked swelling and degeneration of the mitochondria of the cerebral cortex occurs. Cataracts are formed. There are significant increases in the plasma levels of triglycerides and cholesterol. Oxidative stress, as evaluated by mitochondrial damage and mortality, can be prevented by treatment with glutathione esters or ascorbate. This model offers an approach to study of various functions of glutathione, and may be applied to evaluation of the efficacy of compounds in preventing oxidative stress.

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