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MINI-REVIEW

Mitochondrial Glutathione

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Abstract—Many functions of mitochondrial GSH are significantly different from those of cytosolic GSH. This review considers the peculiarity of functions of mitochondrial GSH and enzymes of its metabolism, especially glutathione peroxidase 4, glutaredoxin 2, and κ-glutathione transferase.

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Data are currently accumulating on the specificity of mitochondrial glutathione (mGSH) and significant functional differences between it and cytosolic glutathione (cGSH).

Glutathione is synthesized solely in cytosol, from where it is transferred into other cellular compartments. GSH transport can be conventionally subdivided onto intracellular, intercellular (from astrocytes to neurons [1]), and interorgan (from liver to kidneys and other organs [2]). In the first case, GSH is carried from cytosol across the inner membrane into the mitochondrial matrix where, as a result, 10-15% of total cellular GSH is found, but its concentration is as high as in cytosol (5-10 mM in rat liver), which is required for defense of these organelles. There is no back transport of GSH into the cytosol [3-5]. Cytosolic GSH is exhausted with the inhibitor of its synthesis buthionine sulfoximine much more rapidly than mitochondrial GSH ($T_{0.5}$ values are 2 and 30 h, respectively) [4].

In liver and especially kidney mitochondria dicarboxylate and oxoglutarate carriers are active, which mediate electroneutral exchange of GSH for phosphate and dicarboxylates (GSH is an anion at physiological pH). The oxoglutarate carrier is expressed in heart and brain [6, 7]. However, the tissue specificity of the carrier expression is studied insufficiently.

GSH deficiency results in the spread of damage to mitochondria, which is lethal for newborn rats and guinea pigs unable to synthesize ascorbic acid. The lethal and other possible effects of GSH insufficiency are prevented by GSH esters and ascorbate. Ascorbate and GSH function together in the defense of mitochondria against oxidative damage [8].

As compared with mitochondria of male rats, mitochondria of female rats generate a twofold lower quantity of H_2O_2 and contain twice as active glutathione peroxidase and higher concentration of GSH. Estrogens activate antioxidant enzymes through their receptors, mitogen-activated protein kinase, and NF-κB. This is likely to be an explanation of the longer lifetime of females [9, 10]. These results are extremely interesting; therefore, they need confirmation and further experiments.

GSH transport into mitochondria of hepatocytes is characterized by its dependence on the membrane fluidity. On chronic injections of ethanol, cholesterol is deposited in the inner mitochondrial membrane and decreases the membrane fluidity, which selectively lowers the mGSH concentration because of disorders in the cGSH transport into mitochondria. This results in a progressing damage to the liver [6]. Overexpression of Mn-superoxide dismutase prevents the exhaustion of mGSH, and this indicates the dependence of these events on oxidative stress. Injection of N-acetylcysteine increases the GSH content in cytosol but not in mitochondria, and S-adenosylmethionine (ademethionine) and taurodeoxycholate supply the mGSH content. This is consistent with the therapeutic use of ademethionine and ursodeoxycholate in clinical hepatology [11]. Increasing the fluidity of mitochondria recovers the GSH transport. Injection of ethanol increases hepatotoxicity and sensitizes to tumor necrosis factor-α, which induces the cell death, whereas the above-mentioned preparations protect

Abbreviations: cGSH) cytosolic glutathione; mGSH) mitochondrial glutathione.

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against it [6]. Hypoxia stimulates generation of reactive oxygen species in hepatocytes, exhausts GSH in both cytosol and mitochondria, and increases oxidative damage that can be prevented by inhibitors of the respiratory chain complexes I and II. Selective exhaustion of mGSH with (R,S)-3-OH-4-pentenoate with preserved cGSH sensitizes hepatocytes to hypoxia due to increase in the level of reactive oxygen species, and the recovery of GSH concentration by its ethyl ester or inhibition of the e^- current in complexes I and II saves hepatocytes from such an exposure [12]. Thus, just mGSH controls survival under conditions of oxidative stress.

The inhibitor of GSH synthesis buthionine sulfoximine considerably decreases the cGSH level in different tissues, but weakly affects the mGSH level [8] and survival of neurons [13]. A decrease in the level of both cGSH and mGSH induced by injection of ethacrynic acid inhibits the respiratory complexes [13] and causes degeneration of neurons [14]. Hence, mGSH is especially important for survival of neurons [2]. Selective exhaustion of mGSH with ethacrynic acid in astrocyte culture has been described. The content of mGSH was reported to decrease in focal brain ischemia, and a preliminary injection of GSH monoethyl ester reduced the infarction size. The mGSH may play an important role in brain stroke [15], but this is debatable [16].

Mitochondrial κ-glutathione transferase protects mitochondria, but particular mechanisms of this protection are unclear [17]. The most important enzymes of GSH metabolism in mitochondria are glutathione peroxidases, and their role is especially high because of the absence of catalase in mitochondria. Glutathione peroxidase 1 functions in both cytosol and mitochondria [3]. Glutathione peroxidase 4 (23 kD) possesses a signaling peptide and is carried by the membrane potential into mitochondria, where it is shortened to 20 kD (non-mitochondrial glutathione peroxidase 4 has the same molecular weight). Overexpression of mitochondrial glutathione peroxidase 4 protects different cells against apoptosis caused by various deleterious exposures: additions of H_2O_2, oxidized phospholipids (eicosatetraenoate 15-hydroperoxides, *tert*-butyl, linoleate, phosphatidylcholine, or cholesterol), respiratory chain inhibitors (rotenone, KCN); exhaustion of glucose; by deoxyglucose, staurosporin, etoposide, actinomycin D, and ultraviolet [18]. Overexpression of mitochondrial glutathione peroxidase 4 inhibits production of organic hydroperoxides and loss of the membrane potential and integrity of the plasma membrane, preventing a decrease in the synthesis of ATP and cell death, whereas glutathione peroxidase 1 and other antioxidant enzymes (thioredoxin peroxidase, Cu/Zn- and Mn-superoxide dismutase, catalase, and peroxiredoxins) only partially decrease apoptosis induced by different stimuli [18].

The high protective efficiency of glutathione peroxidase 4 seems to be associated with its direct action on membrane phospholipid hydroperoxides, whereas glutathione peroxidase 1 and other antioxidant enzymes act only after lipid hydroperoxides are released by phospholipase A_2, the affinity of which for them is 10^4 times lower than the affinity of glutathione peroxidase 4 [19, 20]. Mitochondrial but not cytosolic glutathione peroxidase 4 prevents the release from mitochondria of cytochrome *c* and the subsequent activation of caspase 3. This effect is not associated with changes in expression of Bcl-2-bound proteins [21] and is explained by the normal location of cardiolipin as a complex with cytochrome *c* in the inner mitochondrial membrane. Cardiolipin is required for folding and activity of many mitochondrial proteins: F_0F_1-ATPase, adenine nucleotide translocase, cytochrome *c*, and complexes I, III, and IV. Oxidative stress leads to generation of cardiolipin hydroperoxide that is released from its complex with cytochrome *c* which leaves mitochondria and enters the cytosol [18, 22, 23]. The majority of authors consider the produced permeability transition pore to be a complex consisting of adenine nucleotide translocase of the inner membrane, porin of the external membrane (voltage-dependent anionic channel), and cyclophilin D of the mitochondrial matrix [24-26].

Inasmuch as glutathione peroxidase 4 reduces *in vitro* cardiolipin hydroperoxide into hydroxycardiolipin, overexpression of glutathione peroxidase 4 can lower apoptosis by this mechanism [19, 27]. Thus, mitochondrial glutathione peroxidase 4 inhibits cytochrome *c* release from mitochondria and the subsequent apoptosis, but fails to suppress lipoxygenase and cyclooxygenase. Conversely, cytosolic glutathione peroxidase 4, which metabolizes lipid hydroperoxides, does not cause apoptosis, but inhibits 5-, 12-, and 15-lipoxygenases, prevents expression of cyclooxygenase 2, and, therefore, decreases the biosynthesis of leukotrienes and prostanoids and inhibits inflammation. As a result, the two glutathione peroxidases 4 perform different functions in signal transmission, inflammation, and apoptosis. And, respectively, cardiolipin hydroperoxide is a signaling molecule for cytochrome *c* release and apoptotic death, whereas lipid hydroperoxides act as activators of lipoxygenase and cyclooxygenase [18].

Reactions of thiol–disulfide exchange, including protein glutathionylation, occur in both mitochondria and cytosol. These reactions are catalyzed by small (M_r = 10-24 kD) soluble and specific glutaredoxin/thiol transferases, which directly transmit reducing equivalents from GSH during both dithiol and one-electron reduction and also produce mixed disulfides [28]. The recently discovered 15-kD isoform of glutaredoxin 2 is especially important because of vulnerability of mitochondria under oxidative stress [3, 28, 29], which partially inactivates cytosolic/nuclear glutaredoxin 1, while glutaredoxin 2 retains its activity [30]. The catalytic activity of glutaredoxin 2 is lower than the glutathione peroxidase activity

but comparable with that of thioredoxin reductase. Overexpression of glutaredoxin 2 protects cells against H_2O_2-mediated damage of the mitochondrial transmembrane potential. Glutaredoxin 2 can also accept electrons from both GSH and thioredoxin reductase [28].

Under conditions of oxidative stress, oxidation of mGSH increases the production of $O_2^{\cdot-}$ by respiratory chain complex I (and to lesser degree by other respiratory complexes). This occurs due to generation of mixed disulfides during interaction of the accumulating GSSG with redox-active thiols of 75- and 51-kD-subunits of complex I. The duration and degree of reversible glutathionylation are regulated by thioredoxin or glutaredoxin and the redox-state of the mGSH reserve. Reduction of mixed disulfides normalizes the production of $O_2^{\cdot-}$. A similar phenomenon can be also observed on intact mitochondria [31]. Following V. P. Skulachev [32], the authors suggest that H_2O_2 produced from $O_2^{\cdot-}$ is released into the cytoplasm and transmit the signal about the redox-state of the mGSH reserve into the other compartments of the cell [31]. This can be realized with involvement of the H_2O_2-stimulated protein kinase C, NF-κB, platelet-derived growth factor, Jun N-terminal kinase, Ras, and p53. Tumor necrosis factor-α is known to induce oxidation of mGSH and increase the production of reactive oxygen species in mitochondria [33].

The significance of mitochondrial glutathione reductase is less investigated. In the cytosol of brain astrocytes, the GSH level and activities of glutathione reductase, glutathione peroxidases, and glutathione transferases are considerably higher that in neurons, but in mitochondria of neurons all these parameters are higher. Obviously, this favors the defense of neurons against toxic agents [34]. Adenovirus transfer of the gene increases tenfold the activity of mitochondrial glutathione reductase and sevenfold the activity of cytosolic glutathione reductase, which considerably improves the cell resistance to butyl hydroperoxide [35].

We have found no reports concerning mitochondrial γ-glutamyl transferase in the PubMed Database.

Thus, significant differences are revealed between mGSH and cGSH and enzymes of their metabolism:

– mGSH can be selectively exhausted;

– upon exhaustion, mGSH recovers considerably more slowly;

– oxidation of mGSH is associated with an increase in superoxide production by respiratory chain complex I;

– functions of glutathione peroxidase 4 are different in mitochondria (inhibition of cytochrome *c* release and the subsequent apoptosis) and cytosol (a decrease in the synthesis of leukotrienes and prostanoids, inhibition of inflammation);

– mitochondrial glutaredoxin 2 is significantly more resistant than cytosolic/nuclear glutaredoxin 1;

– under conditions of oxidative stress mGSH is much more vulnerable, especially in newborns;

– mGSH is necessary for cell survival under severe conditions. These data indicate the peculiarity of functions of mitochondrial GSH and enzymes of its metabolism, especially such specialized enzymes as glutathione peroxidase 4, glutaredoxin 2, and κ-glutathione transferase.

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