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Roles of Sulfur Metabolism and Rhodanese in Detoxification and Anti-Oxidative Stress Functions in the Liver: Responses to Radiation Exposure

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Organisms must confront various environmental stresses. The liver is central to protecting against such stresses in mammals, and it has many detoxification and anti-oxidative stress functions. Radiation is a source of oxidative stress and is known to affect the liver and induce anti-oxidative responses. The detoxification enzyme rhodanese, which is also called thiosulfate sulfurtransferase (TST), has been demonstrated to be induced in the liver in response to radiation. Cyanide detoxification is a function of the liver, and rhodanese is a key enzyme involved in sulfur metabolism in that detoxification. Though the anti-oxidative stress system in which sulfur molecules such as thiol compounds are involved has attracted attention as a defense against radiation, detoxification enzymes may have other roles in this defense. Understanding how these functions are affected by alterations of sulfur metabolism (including thiol compounds) after irradiation would help uncover their roles in defense against cancer and other deleterious health effects, as well as environmental stress responses. This article reviews the roles of sulfur-related metabolism in oxidative stress regulation and detoxification for recovery from liver damage after radiation exposure, with particular attention to recent findings of sulfur-related enzymes such as rhodanese, which is unique in sulfur metabolism.

MeSH Keywords: **Liver • Oxidative Stress • Radiation • Sulfur Compounds • Thiosulfate Sulfurtransferase**

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Background

Environmental stresses have always been a source of mortality, and ancient organisms evolved the ability to fight such stress. Mammals have evolved diverse stress-protective systems involving low-molecule substances or enzymes [1]. The liver is the main organ for stress response [2–4]. It has many kinds of enzymes and systems against stresses. Among them, there are many for anti-oxidative stress regulation (e.g., GSH, superoxide dismutase, and catalase) and they maintain a proper reducing condition. Oxidative stresses are induced by internal metabolic reactions or external stressors, and many anti-oxidative stress systems in the liver are adapted to cope with reactive oxygen species. Radiation causes oxidative stress and is known to induce anti-oxidative reactions in the liver. It has been demonstrated by proteomic analysis that rhodanese, also called thiosulfate transferase (TST), is induced in the liver after long-term, low-dose radiation exposure [5]. Rhodanese is the main enzyme in sulfur metabolism for cyanide detoxification. Sulfur metabolism is important not only in detoxification but also in anti-oxidative stress systems. As environmental radiation stresses were encountered, organisms developed resistance to environmental radiation. On the earth, organisms have been exposed to radiation from space (originally from solar particle events or galactic cosmic radiation) or radioactive isotopes (e.g., U, Th, and Rn) from natural sources (e.g., rocks or soils) [6]. They must have acquired their protective abilities against radiation during their evolution.

Since cyanide is highly toxic, it is likely that its detoxification has coevolved with and been coopted by other detoxifying systems. This detoxification system is also likely related to anti-oxidative stress functions. Understanding the role of sulfur metabolism after irradiation, including the role of detoxification enzymes such as rhodanese, will lead to potential medical applications.

Sulfur-Related Regulatory Systems as an Intracellular Defense Against Irradiation

Systems that respond to radiation are thought to defend against other environmental stresses or maintain homeostasis. Anti-oxidative stress systems, which are inducible even by low-dose radiation exposure, have been thoroughly researched. In the oxidative stress response, the low-weight molecule glutathione (GSH) plays an important role. Sulfhydryl(thiol)-containing compounds such as GSH are widely known to protect against radiation. For example, N-acetylcysteine (NAC), which is a thiol-reducing agent, induces an increase of GSH in the liver and reduces liver damage after irradiation [7]. Promoting increases in the GSH content in intracellular metabolism is also important. GSH levels in cells are regulated by *de novo* GSH synthesis and the regeneration cycle [8], and low-dose radiation induces GSH biosynthesis [9]. In mouse liver, 0.5 Gy gamma radiation appears to immediately induce GSH, predominantly via recycling and not through *de novo* production, although this is followed by *de novo* production later [9]. It has also been reported that low-dose-rate radiation induces an increase in GSH in mouse liver [8]. The GSH content after irradiation is likely to be regulated by levels of intracellular oxidative stress caused by acute (high-dose-rate) or longer-term (low-dose-rate) radiation. Various radiations, including beta-rays and heavy-ion beams, also seem to mediate cellular oxidative conditions [10,11], although their effects on anti-oxidative stress systems involving intracellular GSH pool regulation need further investigation.

Thioredoxin metabolism is another system that regulates intracellular redox levels and GSH metabolism. Thioredoxin, which is an endogenous thiol-related protein, is induced transiently at the mRNA level even by low-dose irradiation [9,12]. In that case, thioredoxin might contribute to GSH biosynthesis by supplying cysteine to the *de novo* pathway. It has also been reported that metabolic regulation of thioredoxin is related to rhodanese [13,14]. We have demonstrated that rhodanese is induced by long-term, low-dose-rate radiation exposure, and that induction is observed 485 days after the beginning of continuous irradiation [5]. While rhodanese has been investigated as the main enzyme in cyanide metabolism, this kind of long-term induction might be related to its anti-oxidative functions in cooperation with other anti-oxidative molecules such as thioredoxin.

Rhodanese Involvement in Mitochondrial-Related Sulfur Metabolism in Response to Stress

Rhodanese is a mitochondrial protein. Though rhodanese can be induced by external factors such as radiation (Table 1), its induction might also be related to mitochondrial function. We have analyzed the gene expression in mouse kidneys after low-dose-rate, long-term irradiation. We detected alterations in expression in mitochondria-related genes such as the subunit gene (*Ndufb9*) of a protein complex that forms a part of the mitochondrial respiratory chain [15]. Nandi et al. have demonstrated that one of the isoforms of rhodanese functions as thioredoxin oxidase *in vitro* [13]. They suggested a role for the rhodanese isoform in the detoxification of intramitochondrial oxygen free radicals. Indeed, restoration of rhodanese activity is dependent on thioredoxin in cells [14]. Although radiation induces mRNA expression of thioredoxin, the effects of low-dose radiation on thioredoxin function remain to be determined. In addition, mitochondrial function seems to be tightly related to radiation effects, including the alteration of gene expression in kidneys, as mentioned above. Radiation-induced

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Table 1. Sulfurtransferase inducible factors (*in vivo* in livers).

Sulfurtransferases	Inducible factors	Induced levels	Ref.
MPST, CST	Diallyl disulfide	Enzyme activity	[23]
Rhodanese (TST)	Resveratrol	Gene expression	[24]
	α-Lipoic acid	Enzyme activity	[25]
	Phellinus linteus polysaccharide extracts	Protein expression	[30]
	Long-term low-dose irradiation	Protein expression	[5]

damage to mitochondria by long-term irradiation or radiation-induced mediation of ROS production in mitochondria might contribute to persistent oxidative stress [16,17].

Evolutionary Relationship Between MPST and Rhodanese

3-mercaptopyruvate sulfurtransferase (MPST) is a protein closely-related to rhodanese. As MPST is a cytoplasmic and mitochondrial protein, it seems to collaborate with rhodanese in cyanide detoxification and the maintenance of mitochondrial function [18]. The recent development of MPST knockout mice should promote analysis of its function [19]. Since rhodanese and MPST are likely to cooperate with each other, double-knockout mice are possibly lethal [19]. Cyanide detoxification is performed using these sulfurtransferases. These enzymes seem to function principally in metabolism of sulfur compounds [18]. Responses against external stresses seem to have been complemented mutually among them in the process of evolution and have developed new stress response systems. It might be natural that rhodanese, a cyanide-detoxifying enzyme, is also related to anti-oxidative stress systems, taking into consideration that sulfur metabolism contributes to both anti-oxidative function and cyanide detoxification. Since it has also been demonstrated that MPST has a role in anti-oxidative defense systems [19,20], these enzymes might collaborate here too. Although rhodanese seems to have anti-oxidative functions in mammals, its original function is suggested by its participation in anti-oxidative regulation of intracellular homeostasis in the bacterium *Azotobacter vinelandii*. Deletion of Rhodanese-like protein (RhdA) produces oxidative imbalance in the strain [21,22]. Rhodanese or rhodanese-like proteins are present in all domains of life [18]. Though many analyses have been performed, its complete function remains unclear. However, rhodanese may have a key role in maintaining redox homeostasis in mammals, considering the findings of Nandi et al. as mentioned above [13], although direct evidence of this in mammalian cells has not been reported to the best of our knowledge. From the viewpoint of anti-oxidative stress, the evolution of rhodanese needs further study.

Rhodanese Inducers and Radioprotective Functions

MPST and γ-cystathionase (CST) catalyze the formation of sulfane sulfur-containing compounds in anaerobic L-cysteine metabolism, and rhodanese, along with these enzymes, participates in sulfane sulfur transport [23]. Although MPST, rhodanese, and CST are related enzymes, the regulation of each appears to be specific. These sulfurtransferases are induced at the levels of enzyme activation, gene expression, or protein translation by various factors (Table 1).

Diallyl disulfide (DADS) induces CST and MPST activities [23]. Though rhodanese is not induced by DADS, it has been reported that it is induced by resveratrol [24] and that its activity is promoted by α-lipoic acid (LA) [25]. Resveratrol mitigates radiation-induced damage [26] and has been demonstrated to accumulate in the liver and protect hepatocytes from oxidative stress [27]. It has been observed that LA has radioprotective abilities [28], and the effects of LA on anti-stress responses are considered to be due to its anti-oxidative potential [29]. This evidence suggests that rhodanese inducers are related to radioprotection. Recently, *Phellinus linteus* polysaccharide extract (PLP) was demonstrated to induce rhodanese expression in the liver [30]. PLP, which has hepatoprotective abilities, reduces thioacetamide (TAA)-induced liver fibrosis [30]. The induction of rhodanese by PLP is likely related to the regulation of oxidative stress, considering other proteins induced by treatment with PLP. Moreover, reduction of TAA-induced liver fibrosis by PLP suggests that it mitigates persistent stresses. The administration of resveratrol and LA might also suppress inflammation caused by persistent oxidative stress via rhodanese induction. Our group has demonstrated that low-dose-rate, long-term irradiation induces protein expression of rhodanese in mouse liver [5]. Long-term irradiation causes

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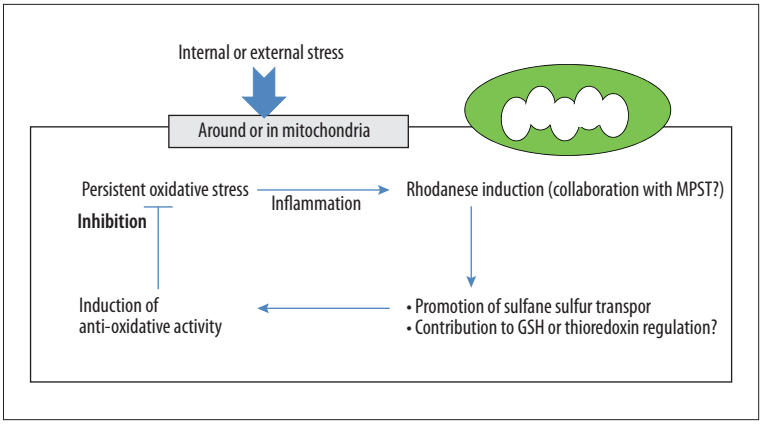

Figure 1. Rhodanese regulation schematic representation.

continuous oxidative stress, possibly resulting in chronic inflammation. Mild oxidative stress might induce rhodanese expression, which mitigates adverse effects such as inflammation.

Medical Relevance of Rhodanese Function

The ability to respond to radiation appears to be necessary for all living things. Continuous inflammatory reactions induce alterations in liver metabolism and may lead to cancer and other pathological conditions [31,32]. Rhodanese function seems to be protective against persistent oxidative stress, including that induced by radiation. As indicated in Figure 1, external (or internal) stresses seem to induce persistent oxidative stresses around mitochondria, leading to rhodanese induction. The induced rhodanese, collaborating with MST, promotes sulfane sulfur, GSH, or thioredoxin regulations, resulting in activation of anti-oxidative stress functions and seems to inhibit the persistent oxidative stresses. Rhodanese has also been reported to be a cancer biomarker candidate [33,34]. Interestingly, the activity of rhodanese has been reported to be reduced in liver tumors, for example in Ehrlich ascites tumor-bearing mice compared to control mice [23]. If its reduction is restored, it might possibly repress tumor growth. As rhodanese inducers include many nutrients (Table 1), it is feasible that its induction could be readily applied clinically. In addition, rhodanese induction might contribute to protection of normal tissues from acute high-dose irradiation in the case of radio-cancer therapy like anti-oxidants, which have been investigated for their protective action [35,36]. In addition, as it has been demonstrated that the reduction of rhodanese expression indicates an increase of oxidative stress and predicts mortality in hemodialysis patients; therefore, its expression might be a prognostic indicator [37].

Conclusions

Since primordial times, organisms have been exposed to ionizing radiation from space or radioactive isotopes in rocks or soil on the earth [6]. During the evolution of life, functions such as those found in rhodanese were developed in very primitive organisms to cope with such stress, and since then they have been adapted to protect against various other stresses in more highly evolved organisms such as mammals. The fact that rhodanese is present in mitochondria suggests its fundamental importance in all living things. Analyses of the effects of exposure to very low-dose irradiation in mice have helped uncover its role in stress response [5]. Its function in anti-oxidative stress systems is likely an evolutionary cooptation. Oxidative stress is related to cancer and metabolic syndrome and influences various processes in cells (e.g., DNA repair pathways [38]). Recent findings by our group showing that obesity-induced oxidative stress mediates the effects of radiation demonstrate the importance of controlling that stress [39].

Understanding anti-oxidative stress regulation is indispensable for current medical advancement. Rhodanese knockout mice have been developed recently by the UC Davis KOMP (Knockout Mouse Project) Repository (*https://www.komp.org/geneinfo.php?geneid=85272*), and analysis of rhodanese function using these mice will uncover new roles of the enzyme and will advance medical science, such as in the detection of biomarkers related to chronic inflammation and other diseases.

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