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COMMENTARY

REDUCED GLUTATHIONE IN COMBINATION WITH SUPEROXIDE DISMUTASE AS AN IMPORTANT BIOLOGICAL ANTIOXIDANT DEFENCE MECHANISM

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Superoxide dismutase (SOD) is a ubiquitous cellular enzyme with an essential function in protecting cells or aerobic organisms against oxidative stress. It catalyses the reaction

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \qquad (1)$$

thereby preventing other reactions of superoxide (O_2^-). In general, however, the chemistry of O_2^- is unremarkable [1], and it is unclear why an efficient removal system should be so important for aerobic life.

Reduced glutathione (GSH) is also widely distributed, with intracellular concentrations typically in the millimolar range. It plays an important role in antioxidant defence by acting in combination with glutathione peroxidases to break down hydrogen peroxide and lipid hydroperoxides [2]. GSH also reacts readily with a wide variety of free radical species, including carbon centred, peroxy, phenoxy and semiquinone radicals [3–5]. It is generally considered, therefore, that GSH also functions as a free radical scavenger and in the repair of radical-mediated biological damage [3–6]. However, any reaction of GSH with a radical species gives rise to the thiyl radical ($GS^\cdot$), so GSH will be successful in detoxication only if subsequent reactions of $GS^\cdot$ are benign.

The reactions of thiyl radicals have been characterized mainly by use of radiolytic techniques [7–11]. In aerobic solution, $GS^\cdot$ can dimerize (a minor reaction), react directly with O_2 to form the thiol peroxy radical (reaction 2) or react with the thiolate anion and then with O_2 (reactions 3 and 4).

$$GS^\cdot + O_2 \rightarrow GSOO^\cdot \qquad (2)$$

$$GS^\cdot + GS^- \rightleftharpoons GS\dot{S}G^- \qquad (3)$$

$$GS\dot{S}G^- + O_2 \rightarrow GSSG + O_2^-. \qquad (4)$$

The thiol peroxy radical decays by a complex route, apparently involving the sulfinyl radical ($RSO^\cdot$) [9], yielding mainly disulfide with the sulfinate ($GS(:O)OH$) and sulfonate ($GS(O_2)OH$) as minor products [12]. However, recent studies indicate that reaction 2 is relatively slow ($k_2 = 3 \times 10^7$/M/sec) [10] and reversible [11], and it is apparent from product analyses and the inhibitory effects of superoxide dismutase [12–15] that, at neutral pH and with GSH in the millimolar range, reaction 3 predominates and the majority of $GS^\cdot$ gives rise to O_2^-.

Thus, radical scavenging by GSH (or any other thiol) initiates a sequence in which one radical ($GS^\cdot$) is replaced by another (O_2^-). This would be beneficial only if the O_2^- was broken down harmlessly. As described below, however, a number of biologically relevant reactions have been described in which radical scavenging by GSH results in a superoxide-dependent chain sequence in which GSH is oxidized to GSSG and O_2 reduced to H_2O_2. If this chain oxidation were to occur intracellularly, GSSG and H_2O_2 would be formed in yields exceeding the initial oxidizing event. This could result in biological damage through subsequent reactions of H_2O_2 or species formed from it, and place an oxidant stress on the cell because of the need to reduce the GSSG. Thus, radical scavenging by GSH could be detrimental rather than protective. Under physiological conditions, however, GSH invariably occurs in association with SOD which, by catalysing reaction (1), prevents this sequence. This association, therefore, should enable GSH to function as an efficient free radical scavenger.

Hypothesis

We propose that an important function of superoxide dismutase is to prevent radical-mediated chain oxidation of GSH, thereby enabling GSH to act physiologically as a free radical scavenger without concomitant oxidative stress to the cell. Through this action, the combination of SOD and GSH plays a significant role in intracellular antioxidant defence.

This mechanism should operate under any conditions where GSH undergoes one-electron oxidation, such as when it auto-oxidizes, a process normally catalysed by metal ions or heme proteins, or interacts with any radical, including O_2^-, generated within the cell. In the following sections we describe examples of SOD protecting GSH against radical-mediated chain oxidation and the combination of SOD and GSH preventing redox cycling reactions.

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Superoxide-mediated GSH oxidation

The simplest chain reaction leading to GSH oxidation that is inhibited by SOD involves reactions 3 and 4 plus reaction 5

$$GSH + O_2^- + H^+ \rightarrow GS^\cdot + H_2O_2 \qquad (5)$$

Several authors have investigated this reaction, both with GSH and other thiols. Reported values for k_5 vary from $<10^3$ to 7×10^5/M/sec [16], although the 10^3–10^4 range is probably most realistic [7, 12]. Although there is still a need to establish an accurate value for k_5, a number of studies have shown that O_2^- can oxidize GSH and other thiols under physiologically relevant conditions. In some cases, O_2 uptake and disulfide formation indicative of a chain reaction have been measured [7, 12, 13, 17]. Wefers and Sies [12] showed that GSH reacts with O_2^- generated by xanthine oxidase and stimulates additional O_2 consumption, and Al-Thannon *et al.* [7] proposed O_2^--dependent chain oxidation of cysteine to explain their radiolytic studies. Using a peroxidase to generate GS or cysteamine radicals, respectively, both Nakamura *et al.* [13] and Svensson and Lindvall [17] measured enhanced O_2 uptake and disulfide formation that was inhibited by SOD, which they attributed to a chain involving reactions 3–5. In other systems, however, a reaction between O_2^- and GSH was not seen [18], and chain lengths and their dependence on factors such as pH and thiol concentration need to be critically assessed.

Nevertheless, there is sufficient evidence in the literature that oxidation of GSH can occur by a radical chain reaction that is inhibited by SOD to suggest that this may be a physiologically important mechanism.

Auto-oxidizable and redox cycling compounds

The most dramatic antioxidant effects of a GSH/SOD combination have been observed with auto-oxidizable compounds. Many classes of chemical compounds undergo one-electron oxidation, including quinols [19], hydroxypyrimidines [20], aromatic amines [21, 22], thiols [23, 24], catecholamines [25, 26], hydrazines [27] and sugars that can form enediols [28]. The reaction may involve direct oxidation by molecular oxygen (which is generally slow) or it may be mediated by a transition metal ion, cytochrome P-450, hemoglobin or a peroxidase:

$$RH_2 + O_2 \xrightarrow{\text{(transition metal)}} RH^\cdot + O_2^- + H^+. \qquad (6)$$

In most cases, oxidation then proceeds via a radical chain reaction involving O_2^- and $RH^\cdot$ (reactions 7–9)

$$RH^\cdot + O_2 \rightarrow R + O_2^- + H^+ \qquad (7)$$

$$O_2^- + RH_2 + H^+ \rightarrow H_2O_2 + RH^\cdot \qquad (8)$$

$$RH_2 + R \rightleftharpoons 2RH^\cdot. \qquad (9)$$

Regardless of the route, the stoichiometry of auto-oxidation is as shown in reaction 10.

$$RH_2 + O_2 \rightarrow R + H_2O_2. \qquad (10)$$

These chain oxidations can be very efficient [20, 26, 28]. SOD inhibits the chain, but oxidation often still proceeds via reactions 7 and 9, with a lag period followed by acceleration as the oxidation product (R) builds up [20, 26].

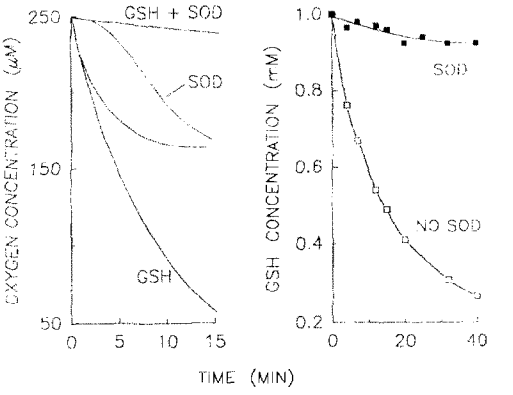

Fig. 1. O_2 consumption and GSH oxidation in solutions of dialuric acid. Dialuric acid (100 μM) in phosphate buffer, pH 7.4, containing 50 μM diethylenetriaminepentaacetic acid (DTPA) was incubated with or without SOD (10 μg/ml) and GSH (1 mM). Results are taken from Ref. 14.

GSH is a good scavenger in these systems.

$$RH^\cdot + GSH \rightarrow RH_2 + GS^\cdot. \qquad (11)$$

However, because $GS^\cdot$ continues to generate O_2 via reactions 3 and 4, the radical chain continues unabated. The difference is that the xenobiotic is recycled and the net reaction becomes

$$2GSH + O_2 \rightarrow GSSG + H_2O_2. \qquad (12)$$

Thus, as observed with hydroxypyrimidine derivatives [14, 15], 1,2,4-triaminobenzene [29], 4-dimethylaminophenol [30] and 4-hydroxyaminoquinoline-1-oxide [31], addition of GSH prolongs O_2 uptake and conversion to H_2O_2. In effect, the xenobiotics catalyse one-electron oxidation of GSH, and radical formation in the presence of the thiol is much greater than in its absence. In isolation, therefore, GSH would be likely to promote toxicity of redox cycling compounds. Furthermore, by reducing compounds such as aromatic disulfides and alloxan, GSH can activate them to undergo O_2^--dependent redox cycling [14, 24]. Dialuric acid and alloxan behave identically in the presence of GSH [14].

However, addition of both SOD and GSH maintains the auto-oxidizable compounds in a reduced form, and there is very little redox cycling and production of H_2O_2 and GSSG. This is illustrated in Fig. 1 for dialuric acid [14]. These results are explicable in terms of SOD preventing chain oxidation involving reaction 8, and GSH, by reducing $RH^\cdot$ and preventing production of R in reaction 7, suppressing the oxidation pathway involving reaction 9. Oxidation then occurs only by slow initiation reactions. GSH and SOD have also been shown to suppress auto-oxidation of divicine [15], isouramil [15] and triaminobenzene [29], and would be expected to have a similar effect with other redox-active compounds.

Radical repair mechanisms and tissue protection

Free radicals can be formed via enzymatic reactions from non-auto-oxidizable substances such as haloalkanes, phenols, nitro compounds and aromatic

amines [32–35]. This "metabolic activation" is thought to be involved in the toxicity of such compounds [32]. Radicals are also formed when cell constituents are exposed to ionizing radiation. These are frequently carbon centred radicals, which react with oxygen to give peroxy radicals. GSH scavenges many of these radicals [3–6, 33–35], regenerating the parent compounds, and often inhibiting peroxidative chains. This process could ameliorate the harmful effects of xenobiotics, and repair by GSH or other thiols is thought to be a major factor in radiation protection [3, 5]. However, the GS· formed by radical scavenging will act as a source of O_2^- (via reactions 3 and 4) so that chain oxidation of GSH or chains involving other O_2^--reactive compounds could still proceed. The effectiveness of GSH-mediated detoxication or radiation protection should, therefore, depend upon the presence of SOD.

Consequences of radical scavenging by GSH in the presence of SOD

Extrapolating to the physiological situation, neither GSH nor SOD alone is capable of preventing the consequences of free radical generation. SOD can destroy only O_2^-, and while GSH efficiently scavenges organic radicals, this reaction may lead to harmful chain reactions. However, inhibition by SOD of chain oxidations permits the benefits of radical scavenging by GSH to be fully expressed. By these reactions, radiation-induced oxidation of tissue components will be repaired and oxidizable compounds will be maintained in their reduced forms. The benefit of the first process is obvious; the second may be advantageous not only in preventing redox cycling but also because many reduced compounds, e.g. quinols, are more easily conjugated and excreted than their oxidation products [36].

One possible disadvantage of maintaining xenobiotics in their reduced form is that they may then be able to reduce ferric complexes, or release iron from ferritin [37], thereby providing a catalyst for the formation of powerful oxidizing agents, such as hydroxyl radical, from H_2O_2. Since H_2O_2 is a product of the GSH/SOD interaction, its removal by catalase or GSH/glutathione peroxidase is a prerequisite for the successful operation of this defensive system.

Conclusion

One-electron oxidations and reductions occur during normal metabolism, and with greater frequency when cells are exposed to oxidizable xenobiotics or radiation. Free radicals are generated in these processes, and it is a reasonable assumption that the high intracellular concentrations of GSH have a protective function in scavenging the radicals and molecular repair. However, such scavenging can set up O_2^--dependent chain production of H_2O_2 and GSSG, which would oxidatively stress the cell. We have described a number of systems in which SOD inhibits this chain. We propose, therefore, that suppression of chain oxidation of GSH is an important physiological function of SOD, and that the combination of SOD and GSH constitutes an integral component of cellular antioxidant defence.

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