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Revisiting the reactions of superoxide with glutathione and other thiols

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ABSTRACT

The reaction between GSH and superoxide has long been of interest in the free radical biology. Early studies were confusing, as some reports suggested that the reaction could be a major pathway for superoxide removal whereas others questioned whether it happened at all. Further research by several investigators, including Helmut Sies, was required to clarify this complex reaction. We now know that superoxide does react with GSH, but the reaction is relatively slow and occurs mostly by a chain reaction that consumes oxygen and regenerates superoxide. Most of the GSH is converted to GSSG, with a small amount of sulfonic acid. As shown by Sies and colleagues, singlet oxygen is a by-product. Although removal of superoxide by GSH may be a minor pathway, GSH and superoxide have a strong physiological connection. GSH is an efficient free radical scavenger, and when it does so, thiyl radicals are generated. These further react to generate superoxide. Therefore, radical scavenging by GSH and other thiols is a source of superoxide and hydrogen peroxide, and to be an antioxidant pathway, there must be efficient removal of these species.

1. Introduction

I first met Helmut Sies at a 1983 EMBO workshop on Oxidative Damage and Related Enzymes, held in Frascati, just outside Rome. I have fond memories of this meeting, partly for the excellent food and ambience of the surrounding countryside, but more particularly as my first introduction to people like Helmut who were pioneering the free radical field. As illustrated in this special issue of ABB, Helmut Sies has made an enormous contribution to redox biology, in fields as diverse as the biological chemistry of carotenoids, glutathione and singlet oxygen, to formulating concepts of oxidative stress and defining what we mean by antioxidant. This article is based on one aspect of Helmut's work that had particular impact on my research, namely the reaction of superoxide with glutathione. But I would first like to mention some of his earliest work, carried out with Britton Chance and Nozomu Oshino in the early 1970s [1–3], in which they used the spectral characteristics of the redox states of catalase to show that hydrogen peroxide is produced during normal metabolism. Using this technology, they were able to estimate H_2O_2 production rates in liver tissue, and steady state concentrations in the nanomolar range. These data remain some of the best quantitative information available and are still widely quoted. However, I believe that the methodology used by Sies and colleagues has been underutilized and may have a place in today's efforts to clarify cellular functions of H_2O_2. The development of fluorescent probes and their use in live cell imaging has led to considerable advances in our understanding of redox metabolism. But powerful as these techniques are, quantification remains challenging. Measuring the redox state of catalase in cells or tissues may well provide complementary information.

The focus of this article relates to a 1983 paper by Sies and Heribert Wefers on the reaction of superoxide with GSH in which they showed that one of the products is singlet oxygen [4]. At that time there was considerable interest in identifying the biological molecules that react with superoxide radicals and establishing which of these reactions contribute to or protect against superoxide toxicity. We were interested in GSH as a free radical scavenger, and the fate of the glutathionyl radicals (GS•) that are produced in the scavenging reaction. As discussed below, these go on to generate superoxide, so it was important to understand the fate of superoxide in such systems. This article describes what we now know about superoxide thiol interactions and how this relates to Sies's earlier findings.

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2. GSH oxidation by superoxide

When we started our investigation [5], several investigators including Wefers and Sies had already shown that superoxide reacts with GSH. However, there was considerable disagreement about whether the reaction was fast enough for GSH to be a major physiological target or was much slower and would therefore be insignificant in cells containing superoxide dismutase (SOD). Reported rate constants ranged from $>10^5$ $M^{-1}s^{-1}$ (which supported the former conclusion) to 15 $M^{-1}s^{-1}$ (which supported the latter). We decided to investigate the reaction further, to obtain a true estimate of the rate and more quantitative information on the reaction products. What transpired, from our work and others, was that there were methodological flaws in many of the previous kinetic studies, and the actual rate lies between the extremes [5,6].

There is now a consensus that the reaction between superoxide and GSH is relatively slow for a radical reaction, with a rate constant (at pH 7.0) of 200 M^{-1} s^{-1} [6]. This means that even at high mM concentrations, GSH would compete poorly with SOD and the reaction would only be relevant at sites where SOD is limiting. Even then, GSH is about 500 times less reactive than ascorbate, which may be a more likely target. The rate increases with increasing pH, and thiols with lower pK_a are more reactive. For example the rate constant for N-acetylcysteine (pK_a 9.5) is 68 M^{-1} s^{-1} [7]. Proteins with low pK_a cysteine residues should be more reactive, and in agreement with this, the rate constant for inactivation of PTP1B by superoxide is twice that for GSH oxidation [8]. However, this still seems insufficient for PTP1B to be a good physiological target for superoxide [9].

The reaction of superoxide with thiols is complex and several oxidation products have been detected. A key feature is that it consumes oxygen. As illustrated in Fig. 1A&C, if superoxide is generated by the reduction of oxygen by xanthine oxidase/hypoxanthine, oxygen uptake increases in the presence of GSH and this increase is inhibited by SOD. GSSG is the main glutathione oxidation product, and as observed by Wefers and Sies [4] glutathione sulfonic acid (GSO_3H) is also formed. They proposed that this accounts for the additional oxygen consumption. However, the mechanism is more complex and this is only part of the explanation [5].

In essence, GSH is first oxidized by superoxide (either via reactions 1–3 or reaction 4 in Scheme 1, as discussed in more detail below) to generate the GS•. Under typical physiological conditions (mM GSH and with oxygen present) the most favorable reaction of GS• is with the thiolate anion (GS^-) to form the disulfide radical anion ($GSSG^{\bullet-}$) (reaction 6) [10,11]. $GSSG^{\bullet-}$ is probably the strongest reductant produced in biological systems [12] and reacts with oxygen irreversibly at a near diffusion-controlled rate to produce GSSG and reduce the oxygen to superoxide (reaction 7). Thus, superoxide is regenerated and the sequence of reactions constitute a radical chain in which oxygen is reduced and GSH is converted to GSSG [5,13]. The superoxide is gradually consumed by competing reactions including dismutation (reactions 11–12) that terminate the chain. The chain tends to be short, with 3–4 GSH oxidized per initial superoxide. Other thiols have also been shown to react with superoxide via a similar mechanism. As shown in Fig. 1C, those with lower pK_a are more reactive [13].

Although reaction 5 is the most favored reaction for GS•, a fraction reacts directly with oxygen (reaction 8) to give the peroxysulfenyl radical (GSOO•). Subsequent reactions of GSOO• lead to the sulfonic acid and other oxygenated species (reaction 9) [14,15]. However, the majority of the GSH oxidized by superoxide (~90%) is converted to GSSG. GSOO• can also break down to give singlet oxygen (reactions 8–10) and is the likely source of the singlet oxygen chemiluminescence observed by Wefers and Sies (Fig. 1B). From other work by Sies and co-workers characterizing its reactivity with thiols [16], any singlet oxygen formed in the superoxide system would be expected oxidise further GSH.

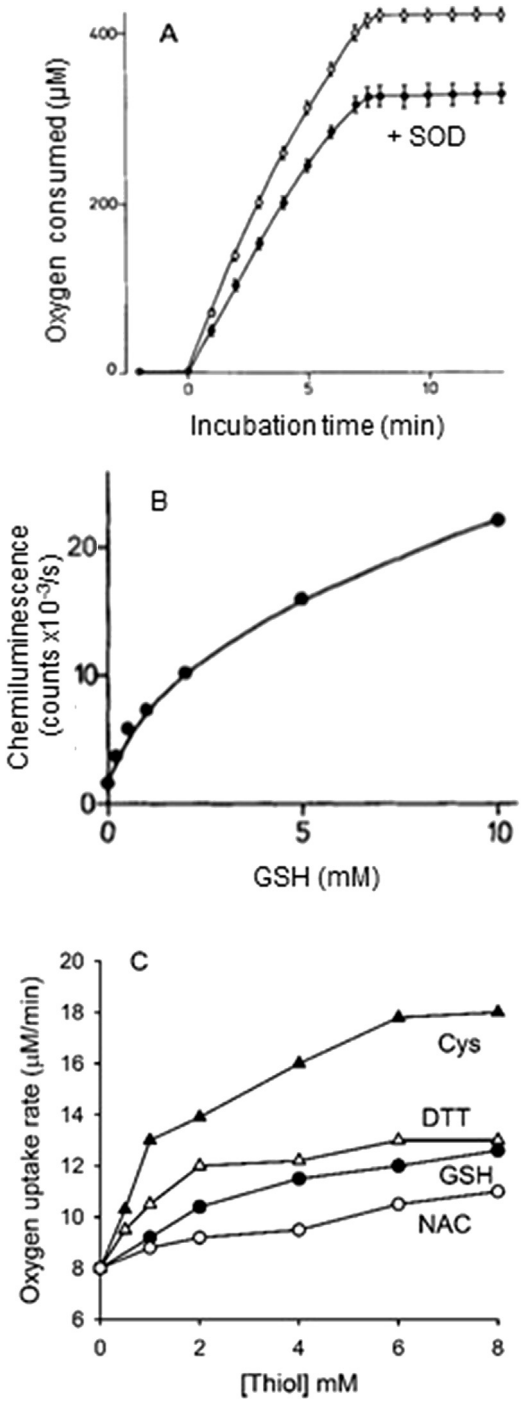

Fig. 1. Superoxide-dependent enhancement of oxygen uptake by a xanthine oxidase system in the presence of GSH. A: Time course in oxygen-saturated buffer pH 7.0 (37 °C) with 700 μM xanthine, 5 mM GSH, catalase and 30 mU/ml xanthine oxidase. B: chemiluminescence at >620 nm. From Wefers and Sies [4] with permission. C: Initial rates of oxygen uptake with GSH (●), Cys (▲), DTT (Δ) and N-acetylCys (○) measured in air at pH 7.4 with 50 μM hypoxanthine, catalase and 8 mU/ml xanthine oxidase. Data taken from [13]. The basal rate represents oxygen consumption by xanthine oxidase and was unaffected by thiol in the presence of SOD.