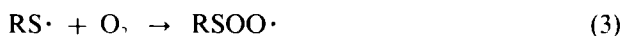


## ESR INVESTIGATIONS OF THE REACTIONS OF RADIATION-PRODUCED THIYL AND DNA PEROXYL RADICALS: FORMATION OF SULFOXYL RADICALS

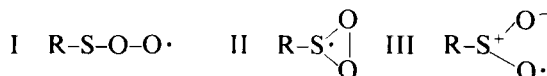
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Thiols are radioprotective agents which act through reaction with radiation produced radicals to form less reactive thiyl radicals.<sup>1</sup> Through this process they eliminate damaging species such as hydroxyl radicals (reaction 1) or repair damage on biomolecules such as DNA by hydrogen atom donation to a carbon centered radical (reaction 2). However in the presence of oxygen two other processes (reaction 3 and 4) occur which may have important biological consequences.



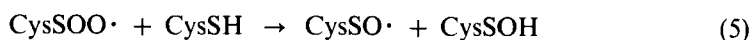
The first process, reaction 3, results in thiol peroxy radical (I) and was proposed by Packer as early as 1963.<sup>2</sup> This reaction has been suggested a number of times since to explain the reactivity of oxygen with thiyl radicals. For example, pulse radiolysis investigations have shown that the reactions of cysteine and glutathione thiyl with oxygen are rapid and form a radical which has a visible absorption at 550 nm.<sup>3</sup> This species has also been identified as the thiol peroxy radical; however, von Sonntag has suggested that such a long wavelength absorption is unknown for other peroxy radicals and has proposed two other possible structures (II,III) for this product.<sup>1</sup> Our ESR work in frozen aqueous solutions has shown that sulfinyl radicals ( $\text{RSO}\cdot$ ) result from the reaction of cysteine and glutathione thiyl radicals with oxygen and although the thiol peroxy radical was proposed as an intermediate, it was not observed.<sup>4</sup>



Pulse radiolysis experiments in aqueous solutions have shown that the rate constants for the second process, reaction 4,<sup>5</sup> are typically several orders of magnitude larger than those for reaction 2.<sup>6</sup> As a consequence, irradiated biological systems are expected to form significant quantities of peroxy radicals even in the presence of thiols. Thus, an understanding of the reactions of thiols with peroxy radicals becomes important to understanding the radioprotective effect of thiols in the presence of oxygen.

Below we summarize our recent work,<sup>7,8</sup> and present new electron spin resonance (ESR) results which may have impact on the radioprotective effect of thiols in the presence of oxygen.

We have reported that the reaction of cysteine thiol radicals with oxygen at low temperatures in dilute frozen aqueous solutions (1 mg/ml in 12 M LiCl or 8 M NaClO<sub>4</sub>) yields the thiol peroxy radical (I).<sup>7</sup> In more concentrated solutions cysteine thiol peroxy radicals react in a biomolecular reaction with thiols (reaction 5) to form sulfinyl radicals (CysSO·) and thus the thiol peroxy radical is not easily observed in these systems.<sup>7</sup>



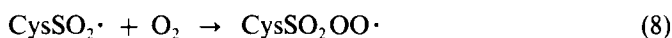
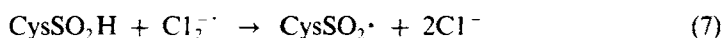
In frozen aqueous solutions the cysteine thiol peroxy radical has a visible absorption with  $\lambda_{\text{max}} = 540 \text{ nm}$  and an ESR spectrum which shows g-value anisotropy typical of a peroxy radical (I).<sup>7</sup>

Our new work shows that anisotropic parallel <sup>17</sup>O couplings of the cysteine thiol peroxy radical are not typical of a carbon centered peroxy radicals we have investigated (see Table 1). A comparison of <sup>17</sup>O couplings found in the table with reported reactivities of peroxy radicals<sup>9</sup> shows that as the terminal oxygen <sup>17</sup>O coupling (and therefore the terminal oxygen spin density) increases the reactivity of the peroxy radicals increases. The comparatively low value for this coupling in the cysteine thiol peroxy radical suggests a quite low reactivity for this species.

Cysteine thiol peroxy radicals was found to photochemically isomerize to sulfonyl radicals at 77 K upon absorption of visible light, reaction 6.<sup>7</sup>



In new work we find that upon annealing to 163 K the sulfonyl radical subsequently reacts with oxygen to form a peroxy radical with <sup>17</sup>O couplings which are typical of a highly reactive peroxy radical (Table 1). This species has been identified as the peroxy sulfonyl radical, CysSO<sub>2</sub>OO· by its separate production from cysteine sulfinic acid in 12 M LiCl as shown in reactions 7 and 8 below.



The ESR spectra and <sup>17</sup>O hyperfine couplings found for CysSO<sub>2</sub>OO· found from both routes are the same.

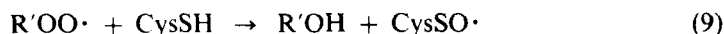
TABLE 1

Anisotropic <sup>17</sup>O Hyperfine Couplings in Peroxy Radicals (R-O<sup>1</sup>-O<sup>2</sup>·) in Frozen Matrices at Low Temperatures

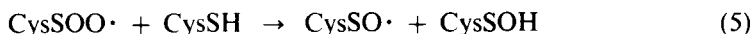
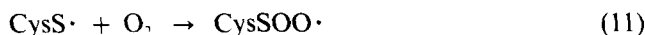
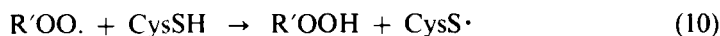
Peroxy Radical Carbon Centered	Matrix	A <sub>1</sub> (O <sup>1</sup> ) <sup>a</sup>	A <sub>1</sub> (O <sup>2</sup> )	Temperature
R-CF <sub>2</sub> OO·	PTFE <sup>b</sup>	4.4 mT	10.7 mT	77 K
CClF <sub>2</sub> CClFOO·	CClF <sub>2</sub> CCl <sub>2</sub> F	4.5	10.5	77
CO <sub>2</sub> HCH <sub>2</sub> OO·	12 M LiCl	5.7	9.8	106
NH <sub>3</sub> <sup>+</sup> CH(CO <sub>2</sub> <sup>-</sup> )CH <sub>2</sub> OO·	12 M LiCl	5.9	9.8	77
CH <sub>2</sub> =CH-CH <sub>2</sub> OO·	12 M LiCl	6.0	9.6	107
CH <sub>3</sub> CH(OO·)CO <sub>2</sub> <sup>-</sup>	12 M LiCl	5.9	9.4	110
Sulfur Centered				
NH <sub>3</sub> <sup>+</sup> CH(CO <sub>2</sub> <sup>-</sup> )CH <sub>2</sub> SO <sub>2</sub> OO·	8 M NaClO <sub>4</sub>	4.5	10.6	77
NH <sub>3</sub> <sup>+</sup> CH(CO <sub>2</sub> <sup>-</sup> )CH <sub>2</sub> SOO·	8 M NaClO <sub>4</sub>	6.4	8.1	77

<sup>a</sup> The perpendicular couplings are less than the experimental linewidth of ca. 1 mT.<sup>b</sup> Olivier, D., Marachi, C. and Che, M., EPR study of temperature dependent rotations of polytetrafluoroethylene chains labeled with peroxy radicals, *J. Chem. Phys.*, **71**, 4688 (1979).

Carbon centered peroxy radicals have been found to react with thiols to form sulfinyl radicals either by reaction 9 or by 10,11 followed by 5.<sup>8</sup>



or



We find that the reaction of peroxy radicals with thiols to form sulfinyl radicals occurs in irradiated frozen water solutions of DNA. In Figure 1A we show the ESR spectra found immediately after irradiation (10 kGy) at 77 K of a 100 mg/ml solution of DNA with oxygen present. At this temperature the major radicals are considered to be DNA base anion and cation radicals. A very small amount of the thymine protonated anion ( $\text{TH}\cdot$ ) is present. Figure 1B shows the formation of DNA peroxy

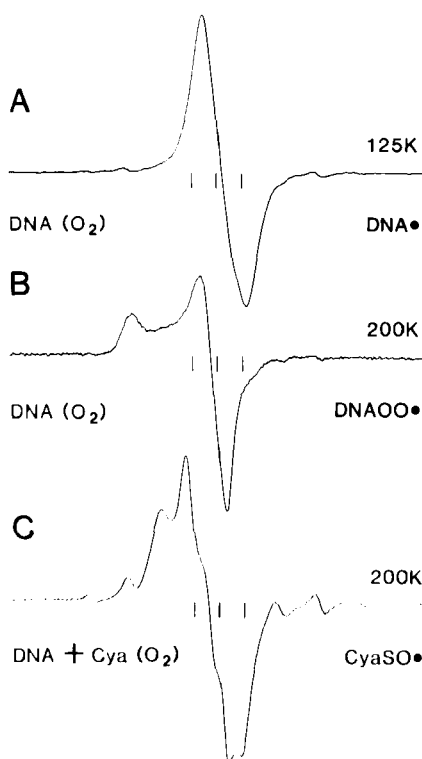


FIGURE 1 The first derivative ESR spectra of a  $\gamma$ -irradiated frozen aqueous solution of DNA (100 mg/ml) in the presence of oxygen. A. Immediately after irradiation and annealing to 125 K. At this temperature mainly DNA base radicals,  $\text{DNA}\cdot$ , are present. B. After annealing to 200 K only DNA peroxy radicals,  $\text{DNAOO}\cdot$ , are found. C. Spectrum found after annealing an identical sample to that in A except with 3 mg/ml cysteamine. In this case the major species present is the cysteamine sulfinyl radical,  $\text{CyaSO}\cdot$ .

radical after annealing the sample in IA to 200 K. Identical treatment of a sample of 100 mg/ml of cysteamine (Cya) gives the same initial spectrum, but on warming to 200 K the major radical present is found to be the cysteamine sulfinyl radical (CyaSO $\cdot$ ), with a small amount of the eight line spectrum of TH $\cdot$ , Figure 1C. These results are considered good evidence that DNA peroxy radicals react with thiols to form sulfinyl radicals.

### References

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