Generation of Free Radicals in Reactions of Ni(II)-Thiol Complexes with Molecular Oxygen and Model Lipid Hydroperoxides

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ABSTRACT

The generation of free radicals from reactions of nickel(II)-thiol complexes with molecular oxygen and model lipid hydroperoxides was investigated by electron spin resonance (ESR) utilizing 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap. Incubation of nickel(II) [Ni(II)] with cysteine in an aerobic environment generated hydroxyl (·OH) radical, which then reacted with cysteine to generate a carbon-centered alkyl (·R) radical. Radical generation was inhibited under a nitrogen atmosphere. Model lipid hydroperoxides, cumene hydroperoxide, and *t*-butyl hydroperoxide enhanced the yield of these radicals and also generated an alkoxyl (·OR) radical. Radical yield decreased by approximately half under a nitrogen atmosphere. Although histidine did not cause radical formation in the reaction between Ni(II) and cumene hydroperoxide under aerobic conditions, the addition of histidine to a mixture containing Ni(II), cysteine, and cumene hydroperoxide under the same experimental conditions increased the yield of ·R radical but lowered the yield of ·OR and ·OH radical adducts. It thus appears that histidine caused the ·OH attack to be more site-specific. Similar results were obtained utilizing *t*-butyl hydroperoxide. Penicillamine or *N*-acetylcysteine yielded similar results except that under aerobic conditions, reaction between Ni(II) and *N*-acetylcysteine without hydroperoxide did not generate a significant concentration of free radicals. Under the same experimental conditions, cystine did not generate any detectable free radicals, suggesting an important role of the -SH group in Ni(II)-mediated free radical generation. The results indicate that free radical generation from the reaction of Ni(II)-thiol complexes and molecular oxygen, and/or lipid hydroperoxides, may play an important role in the mechanism(s) of Ni(II) toxicity and carcinogenesis.

INTRODUCTION

While nickel is a well-recognized carcinogen [1, 2], the underlying biochemical mechanisms remain unclear [3, 4]. Recent studies suggest that these mechanisms

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may involve oxidative DNA damage through free radicals, especially the hydroxyl (·OH) radical [5–13]. The mechanisms of nickel carcinogenicity and toxicity may also involve lipid peroxidation [14–21]. Unlike ferrous ion [Fe(II)], the Ni(II) cation itself does not directly react with O_2, H_2O_2, or lipid hydroperoxides to generate free radicals [12, 21]. However, it has been reported that Ni(II) complexes with certain synthetic oligopeptides, e.g., tetraglycine or glycylglycylhistidine, make Ni(II) reactive with O_2. This reaction results in transitory formation of Ni(III) and degradation of the organic ligand via free radical reactions [22]. Such Ni(II)-peptide complexes are also capable of reacting with H_2O_2 to produce oxygen free radicals [11, 12]. Although the cellular environment may not contain these particular peptide ligands that are known to render Ni(II) redox active in vitro, it does contain a variety of oligopeptides that are capable of forming Ni(II) complexes at physiological pH, and thus may facilitate Ni(II) redox activity in vivo. These oligopeptides include glutathione (γ-L-glutamylycysteinylglycine), carnosine (β-alanyl-L-histidine), homocarnosine (γ-aminobutyryl-L-histidine), and anserine (β-alanyl-3-methyl-L-histidine).

In our most recent study [21], it has been shown that in the presence of Ni(II) all these peptides are able to generate hydroperoxide-derived free radicals from two model hydroperoxides, cumene hydroperoxide (cumene-OOH), and *t*-butyl hydroperoxide (*t*-butyl-OOH). The significance of this finding to nickel carcinogenicity stems from the fact that exposure of cells to Ni(II) may result in the formation of all reactants that are necessary for free radical generation, i.e., Ni(II) complexes and lipid hydroperoxides. Carnosine, homocarnosine, and anserine contain the histidine residue, which has an exceptionally high affinity for Ni(II) and thus enhances Ni(II) binding by peptides and proteins [23, 24]. Glutathione contains cysteine, another strong metal-binding amino acid. Both cysteine and histidine are known to form strong complexes with Ni(II) and serve as major physiological low-molecular weight Ni(II) carriers [25]. Reactivity of the Ni(II)-glutathione system toward hydroperoxides is much higher than that of the remaining three oligopeptides [21]. This signalled a possible high reactivity of Ni(II) complexes with other sulfhydryl (thiol) ligands, such as cysteine and the cysteine-containing oligopeptides, toward hydroperoxides. Therefore, in the present study, we investigated the possible free radical generation in systems with cysteine and two other thiol chelators of Ni(II), *N*-acetylcysteine and penicillamine. Since Ni(II) forms an exceptionally strong ternary Ni(II)-cysteine-histidine complex in vivo [25], free radical generation from model lipid hydroperoxides in the presence of Ni(II), cysteine, and histidine was also investigated.

MATERIALS AND METHODS

1. Materials

Ethanol and phosphate buffer (100 mM, pH 7.2) were purchased from Fisher Scientific (Pittsburgh, PA). DL-Cysteine, DL-penicillamine, *N*-acetyl-L-cysteine, L-histidine, cystine, sodium formate, cumene-OOH, and *t*-butyl-OOH were purchased from Sigma Chemical Co. (St. Louis, MO). Nickel(II) chloride ($NiCl_2 \cdot H_2O$), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Potassium tetraperoxochromate (K_3CrO_8) was prepared by K. Singh [26].

Chelex-100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA). The phosphate buffer was treated with Chelex-100 to remove possible transition metal ion contaminants. DMPO solution was purified using activated charcoal until free radical impurities disappeared as verified by ESR spectroscopy.

2. ESR Measurements

ESR spin trapping methodology [27, 28] was employed for detecting short-lived free radical intermediates. The spectra were obtained using a Varian E3 spectrometer operating at 9.4 GHz with a 100-KHZ modulation frequency. Hyperfine splittings of the spin adduct were measured (to 0.1 G) directly from magnetic field separation using K_3CrO_8 and DPPH as standards. Reactants dissolved in 100 mM phosphate buffer, pH 7.2, were mixed in test tubes in a total final volume of 0.25 ml. The reaction mixture was then transferred to a flat cell (purchased from Wilmad, Buena, NJ) for ESR measurement. The concentrations given in the figure legends are final concentrations. All experiments were carried out at room temperature and under ambient atmosphere except those specifically indicated contrary to this.

RESULTS

1. Free Radical Generation in a Mixture of Ni(II) and Cysteine

The ESR spectrum obtained from a mixture of 1 mM Ni(II), 2 mM cysteine, and 200 mM DMPO under aerobic conditions is shown in Figure 1a. Computer simulation analysis shows that this spectrum is a composite of two spin adduct signals. The signal with hyperfine splittings of $a_N = 16.7$ G and $a_H = 22.5$ G (marked by asterisks) was assigned to a DMPO/·R adduct (·R represents a carbon-centered alkyl radical). This assignment was made based on the known hyperfine splittings of such adducts [29]. The other signal, with hyperfine splittings of $a_N = a_H = 15.0$ G, was assigned to the DMPO/·OH adduct [29–31]. Cysteine or Ni(II) alone did not generate a detectable amount of free radicals (Figs. 1b and 1c), showing that the simultaneous presence of Ni(II) and cysteine was essential for both ·R and ·OH radical generation.

To understand the mechanism of the free radical formation in the reaction mixture containing Ni(II) and cysteine, ethanol, an ·OH radical scavenger, was added to this mixture. As can be noted from Figure 1d, addition of ethanol suppressed the ·OH radical generation and enhanced the ·R radical formation. It is known that reaction between ethanol and ·OH radical produces ethanoyl ($\cdot CHOHCH_3$) radical, which can be trapped by DMPO to generate the DMPO/$\cdot CHOHCH_3$ adduct signal [32, 33]. The DMPO/$\cdot CHOHCH_3$ radical adduct exhibits essentially the same hyperfine splittings as those resulting from DMPO trapping of other carbon-center alkyl radicals [29]. Thus the enhancement of ·R radical generation upon addition of ethanol (Fig. 1d) may be due to the contribution of the DMPO/$\cdot CHOHCH_3$ radical adduct signal. As expected, the addition of another commonly used ·OH radical scavenger, sodium formate, also suppressed the ·OH radical generation and produced predominantly a DMPO/$\cdot COO^-$ adduct signal with hyperfine splittings of $a_N = 15.7$ G and $a_H = 18.7$ G. Since ·R radicals disappeared upon addition of sodium formate

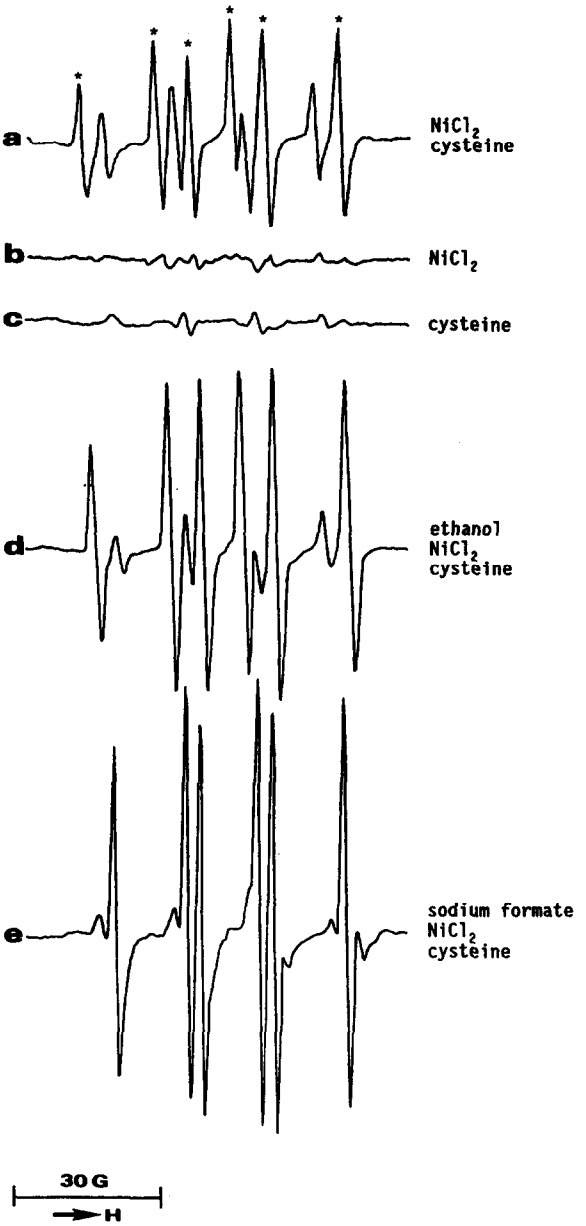

FIGURE 1. (a) ESR spectrum, recorded 45 seconds after reaction initiation, from a pH 7.2 phosphate buffer solution of 1 mM $NiCl_2$, 2 mM cysteine, and 200 mM DMPO. (b) Same as (a) but without cysteine. (c) Same as (a) but without $NiCl_2$. (d) Same as (a) but with 1.6 M ethanol added. (e) Same as (a) but with 1.6 M sodium formate added. The ESR spectrometer settings were: receiver gain, 1.5×10^5; time constant, 0.3 second; modulation amplitude, 1.25 G; scan time, 8 minutes; magnetic field, 3470 ± 100 G. The asterisks indicate the DMPO/·R radical adduct signal.

(Fig. 1e), it can be concluded that the ·R radicals were generated via reaction between cysteine and ·OH radicals. When sodium formate was added, it reacted with ·OH radical in competition with cysteine. Since sodium formate was present at a much higher concentration (1.6 M) than cysteine (2.0 mM), ·OH radicals reacted almost exclusively with formate to generate $\cdot COO^-$ radicals.

To examine the role of molecular oxygen in the free radical generation, the measurements were carried out under nitrogen. The result is presented in Figure 2b. As can be seen in this figure, under a nitrogen atmosphere a mixture containing Ni(II) and cysteine generated a much smaller amount of free radicals (compare with Fig. 2a), showing that molecular oxygen was indeed involved in the free radical generation mechanism. The small amount of radicals detected may be due to the incomplete removal of oxygen. To examine the role of the -SH group in the radical generation, cysteine was replaced by cystine. As shown in Figure 2c, a mixture containing Ni(II) and cystine in the presence of molecular oxygen did not generate a detectable amount of free radicals, demonstrating the essential role of the -SH group in the radical generation.

2. Free Radical Generation in Mixtures of Ni(II), Cysteine, and Hydroperoxides

Cumene-OOH. As shown in Figure 3a, a reaction mixture containing 1 mM Ni(II), 2 mM cysteine, 5 mM cumene-OOH, and 200 mM DMPO generated a

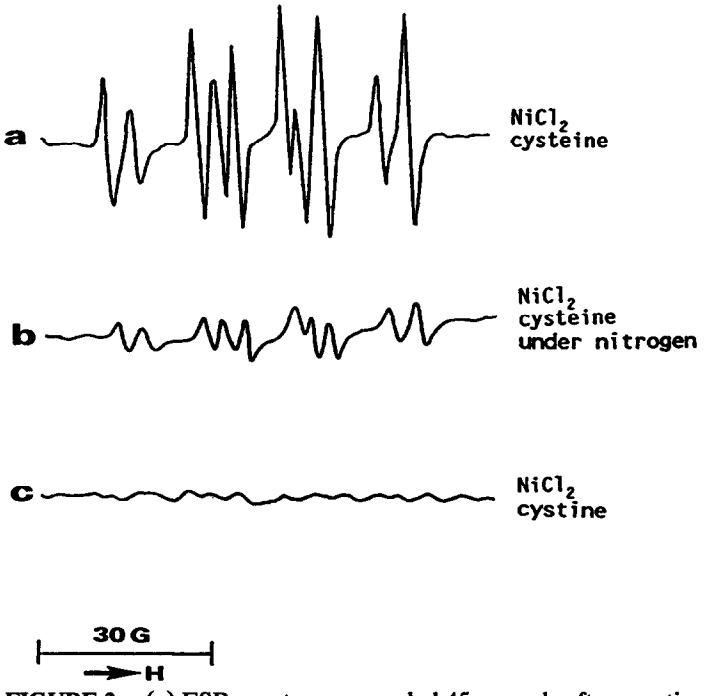

FIGURE 2. (a) ESR spectrum, recorded 45 seconds after reaction initiation, from a pH 7.2 phosphate buffer solution of 1 mM $NiCl_2$, 2 mM cysteine, and 200 mM DMPO. (b) Same as (a) but under nitrogen. (c) Same as (a) but using cystine instead of cysteine. The spectrometer settings were the same as those in Figure 1.

pronounced spin adduct signal. Computer analysis showed that this spectrum consists of three radical adducts. The first one (marked by asterisks) with hyperfine splittings of $a_N = 16.7$ G and $a_H = 22.5$ G was assigned to DMPO/·R. The other signal with hyperfine splittings of $a_N = a_H = 15.0$ G was assigned to a mixture of DMPO/·OH and DMPO/·OR (·OR represents the oxygen-centered cumenyl radical). The hyperfine splitting of DMPO/·OR was $a_N = 15.0$ G and $a_H = 16.2$ G, which was overlapped with those of DMPO/·OH (Fig. 3a). This phenomenon has been previously observed from a similar system [34]. As shown in Figure 3b, reaction between cysteine and cumene-OOH without Ni(II) generated similar radicals but the yield was much lower. As another control, it can be noted from Figure 3c that reaction between Ni(II) and cumene-OOH did not generate any detectable amount of free radicals, again showing that Ni(II) in the absence of thiol did not react with lipid hydroperoxide at a significant rate. Formate caused the generation of DMPO/·COO^- adduct and decreased the yield of DMPO/·R, DMPO/·OR, and DMPO/·OH radicals (Fig. 3d), indicating that a significant portion of ·R and ·OR radicals was generated via ·OH radical-mediated reactions. However, as may be noted from Figure 3d, sodium formate did not completely eliminate the DMPO/·R and DMPO/·OH signals, suggesting that some of the radicals were generated via an ·OH-independent mechanism, perhaps via direct reaction between an Ni(II)-cysteine complex and cumene-OOH. When the same experiment as in Figure 3a was carried out under a nitrogen atmosphere, the yield of the radicals decreased by approximately half (Fig. 3e). When cystine was used in place of cysteine no spin adduct signal was produced (Fig. 3f).

t-Butyl-OOH. The ESR spectrum obtained with *t*-butyl-OOH was similar to that produced by cumene-OOH (Fig. 4a). This spectrum was assigned to a combination of DMPO/·R, DMPO/·OR and DMPO/·OH adducts. Reaction between cysteine and *t*-butyl-OOH in the absence of Ni(II) also generated some spin adduct, although in significantly smaller amounts (Fig. 4b). Mixture of Ni(II) and *t*-butyl-OOH did not generate any detectable radical adduct signal (Fig. 4c). As expected, addition of sodium formate produced predominantly the DMPO/·COO^- signal and decreased the DMPO/·R, DMPO/·OH, and DMPO/·OR adduct signals (Fig. 4d). When the same experiment as that in Figure 4a was carried out under a nitrogen atmosphere, the radical yield decreased by approximately half. No spin adduct signal was observed when cysteine was replaced by cystine (Fig. 4f).

3. Free Radical Generation in Mixtures of Ni(II), Cysteine, Histidine, and Hydroperoxides

Cumene-OOH. As shown in Figure 5a, a reaction mixture containing 1 mM Ni(II), 2 mM cysteine, 2 mM histidine, 5 mM cumene-OOH, and 200 mM DMPO generated predominantly DMPO/·R at a higher yield than that without histidine (Fig. 3a) (note the lower spectrometer gain settings in Fig. 5). Only weak DMPO/·OH and DMPO/·OR adduct signals were observed. Reaction of Ni(II) with histidine and cumene-OOH without cysteine did not generate any radical adduct signal (Fig. 5b), showing that histidine alone did not enhance the reactivity of Ni(II) toward lipid hydroperoxide. Changing the ratio of Ni(II): cysteine:histidine from 1:2:2 to 1:1:1 decreased the overall intensity of the spin adduct signals by about 30% (data not shown).

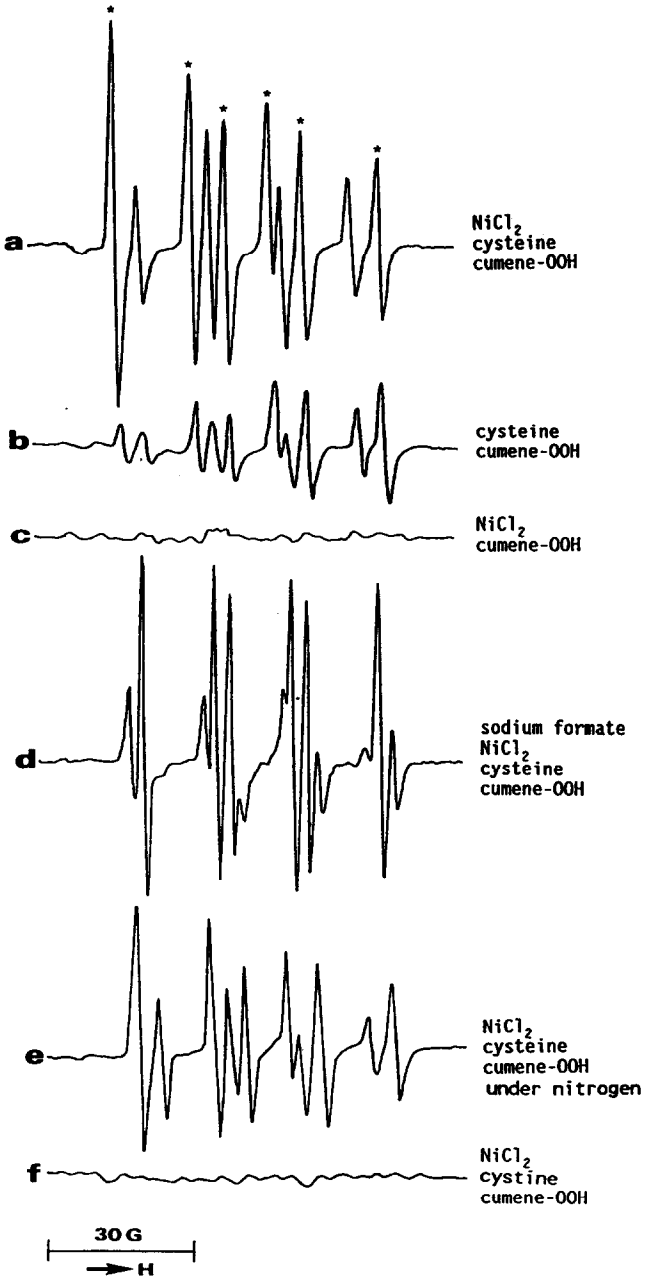

FIGURE 3. (a) ESR spectrum, recorded 45 seconds after reaction initiation, from a pH 7.2 phosphate buffer solution of 1 mM $NiCl_2$, 2 mM cysteine, 5 mM cumene-OOH, and 200 mM DMPO. (b) Same as (a) but without $NiCl_2$. (c) Same as (a) but without cysteine. (d) Same as (a) but with 1.6 M sodium formate added. (e) Same as (a) but under nitrogen. (f) Same as (a) but using 2 mM cystine instead of cysteine. The ESR spectrometer settings were the same as those in Figure 1. The asterisks indicate the DMPO/·R radical adduct signal.

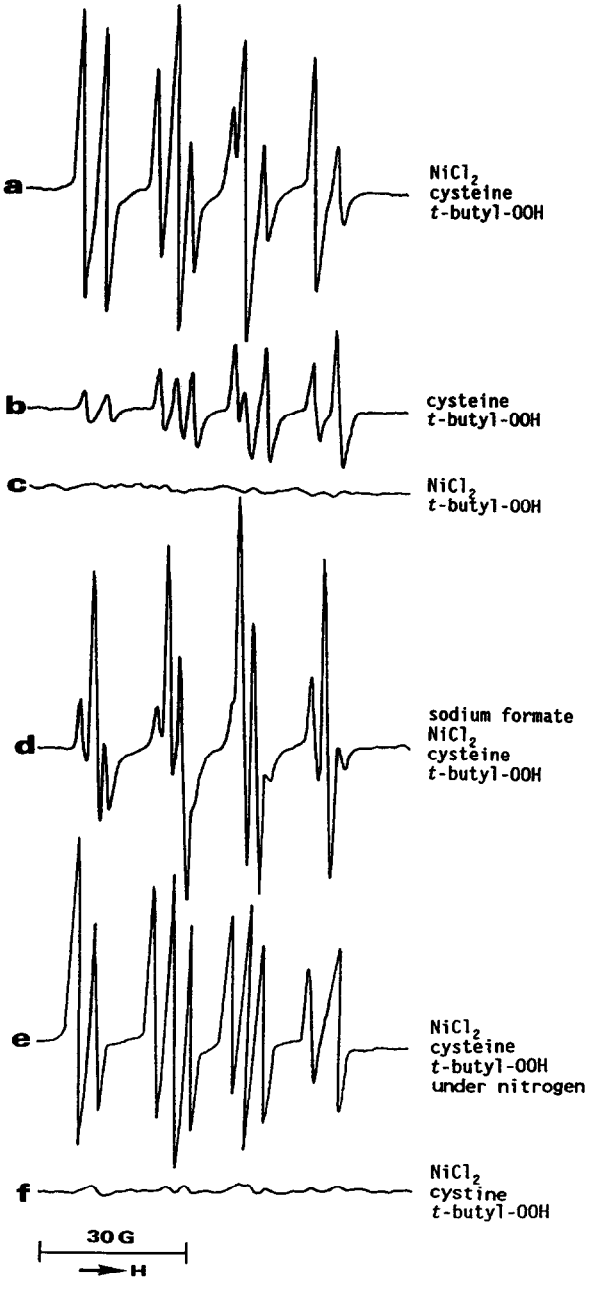

FIGURE 4. (a) ESR spectrum, recorded 45 seconds after reaction initiation, from a pH 7.2 phosphate buffer solution of 1 mM $NiCl_2$, 2 mM cysteine, 5 mM *t*-butyl-OOH, and 200 mM DMPO. (b) Same as (a) but without $NiCl_2$. (c) Same as (a) but without cysteine. (d) Same as (a) but with 1.6 M sodium formate added. (e) Same as (a) but under nitrogen. (f) Same as (a) but using 2 mM cystine instead of cysteine. The ESR spectrometer settings were the same as those in Figure 1.

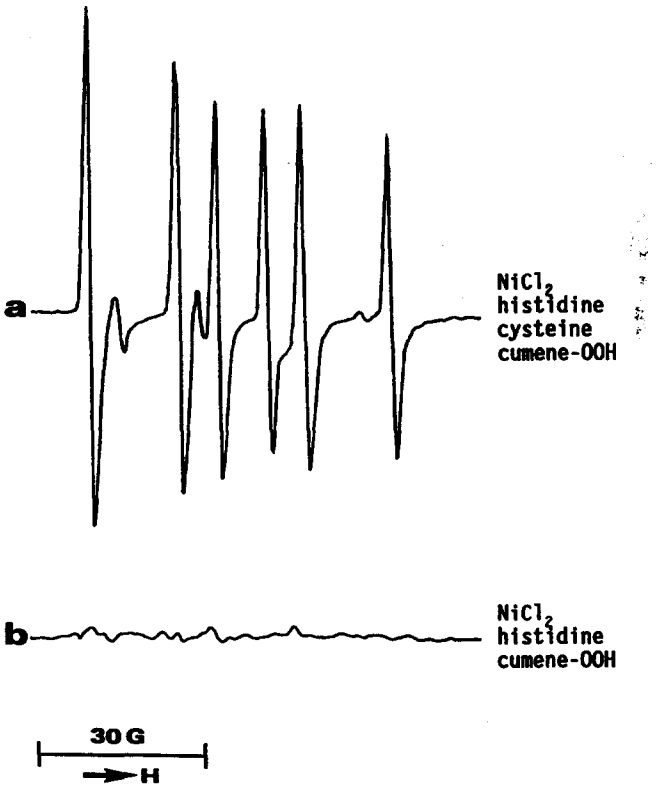

FIGURE 5. (a) ESR spectrum, recorded 45 seconds after reaction initiation, from a pH 7.2 phosphate buffer solution of 1 mM $NiCl_2$, 2 mM cysteine, 2 mM histidine, 5 mM cumene-OOH, and 200 mM DMPO. (b) Same as (a) but without cysteine. The ESR spectrometer settings were the same as those in Figure 1 except that the receiver gain was 8.0×10^4.

t-Butyl-OOH. Essentially the same results as described above were obtained using *t*-butyl-OOH in place of cumene-OOH (Fig. 6). As may be noted from Figure 6a, histidine caused an enhancement of DMPO/·R generation and a decrease in the yield of DMPO/·R, DMPO/·OH, and DMPO/·OR. Similarly, the reaction of Ni(II), histidine and *t*-butyl-OOH did not generate any free radical adduct signal (Fig. 6b). Changing the ratio of Ni(II):cysteine:histidine from 1:2:2 to 1:1:1 decreased the overall intensity of the spin adduct signals by about 30% (data not shown).

4. Free Radical Generation in Mixtures of Ni(II) with *N*-Acetylcysteine, Penicillamine, and Hydroperoxides

Cumene-OOH. As shown in Figure 7a, a reaction mixture containing 1 mM Ni(II), 2 mM penicillamine, 5 mM cumene, and 200 mM DMPO generated a spin adduct signal similar to that observed for cysteine (compare with Figs. 1a and 2a). This spectrum was assigned to a mixture of DMPO/·R, DMPO/·OR, and DMPO/·OH free radical adducts. Likewise, a mixture of Ni(II) and penicillamine without hydroperoxide or a mixture of penicillamine and cumene-

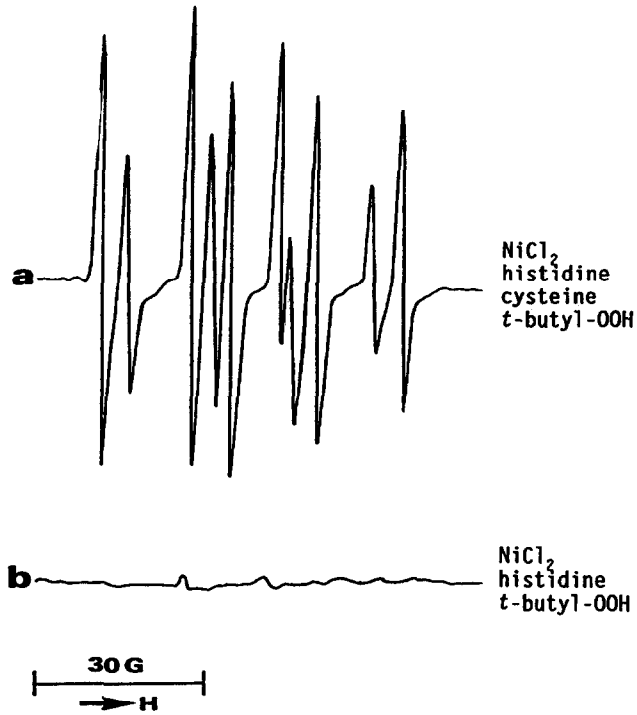

FIGURE 6. (a) ESR spectrum, recorded 45 seconds after reaction initiation, from a pH 7.2 phosphate buffer solution of 1 mM $NiCl_2$, 2 mM cysteine, 2 mM histidine, 5 mM *t*-butyl-OOH, and 200 mM DMPO. (b) Same as (a) but without cysteine. The ESR spectrometer settings were the same as those in Figure 1.

OOH without Ni(II) also generated a small amount of radical adduct signals (Figs. 7b and 7c).

Essentially the same results were obtained when *N*-acetylcysteine was used in place of penicillamine (Fig. 8a). However, unlike in the case of cysteine or penicillamine, a mixture of Ni(II) and *N*-acetylcysteine without hydroperoxide or a mixture of *N*-acetylcysteine and cumene-OOH without Ni(II) did not produce any radical adducts (Figs. 8b and 8c).

t-Butyl-OOH. Replacement of cumene-OOH by *t*-butyl-OOH produced results similar to those described above (Figs. 7d and 7e and Figs. 8d and 8e). Again, unlike in the cases of cysteine and penicillamine, a mixture of *N*-acetylcysteine and *t*-butyl-OOH without Ni(II) did not generate any free radical adduct (Fig. 8e)

DISCUSSION

The overall goal of the present study was to search for Ni(II)-mediated free radical generation in biologically relevant systems. Such systems, among others, contain an abundance of thiol-bearing molecules that may serve as transition metal cation binding ligands. For example, cysteine and glutathione have been reported to form strong Ni(II) complexes at pH 7.4 [23, 35, 36]. The same is true for penicillamine [35].

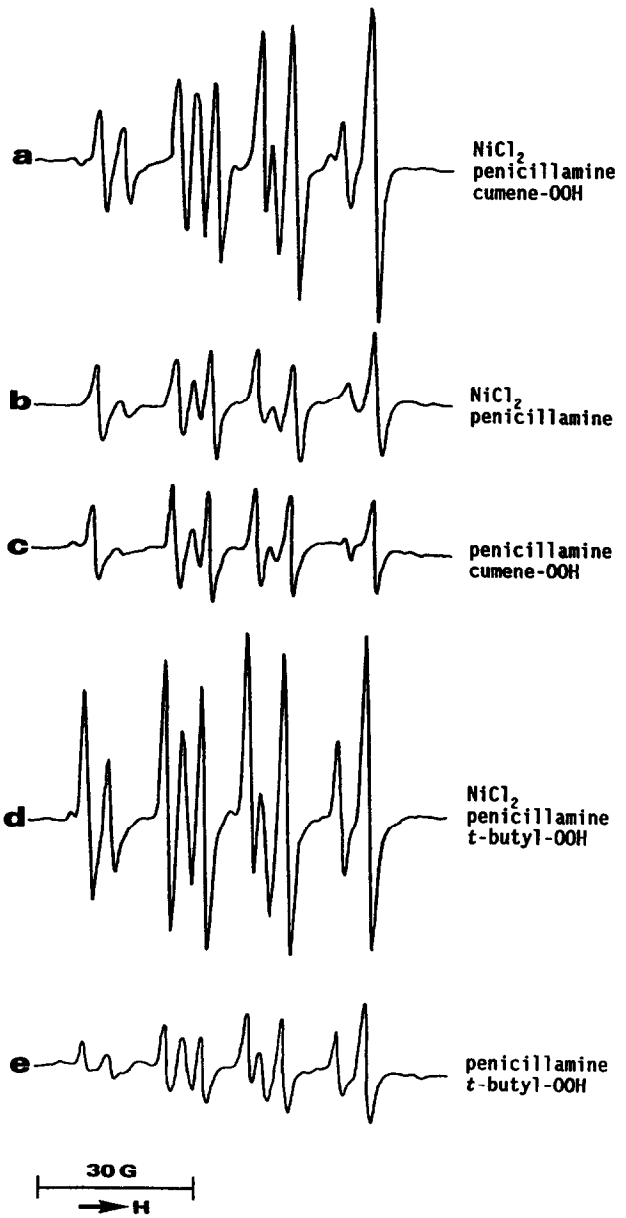

FIGURE 7. ESR spectra, recorded 6 minutes after reaction initiation, from a pH 7.2 phosphate buffer solution of 200 mM DMPO and the following reactants: (a) 1 mM $NiCl_2$, 2 mM penicillamine and 5 mM cumene-OOH; (b) 1 mM $NiCl_2$ and 2 mM penicillamine; (c) 2 mM penicillamine and 5 mM cumene-OOH; (d) 2 mM $NiCl_2$, 2 mM penicillamine and 5 mM *t*-butyl-OOH; (e) 2 mM penicillamine and *t*-butyl-OOH. The ESR spectrometer settings were: receiver gain, 1.0×10^5; time constant, 0.3 second; modulation amplitude, 1.0 G; scan time, 8 minutes; magnetic field, 3470 ± 100 G.

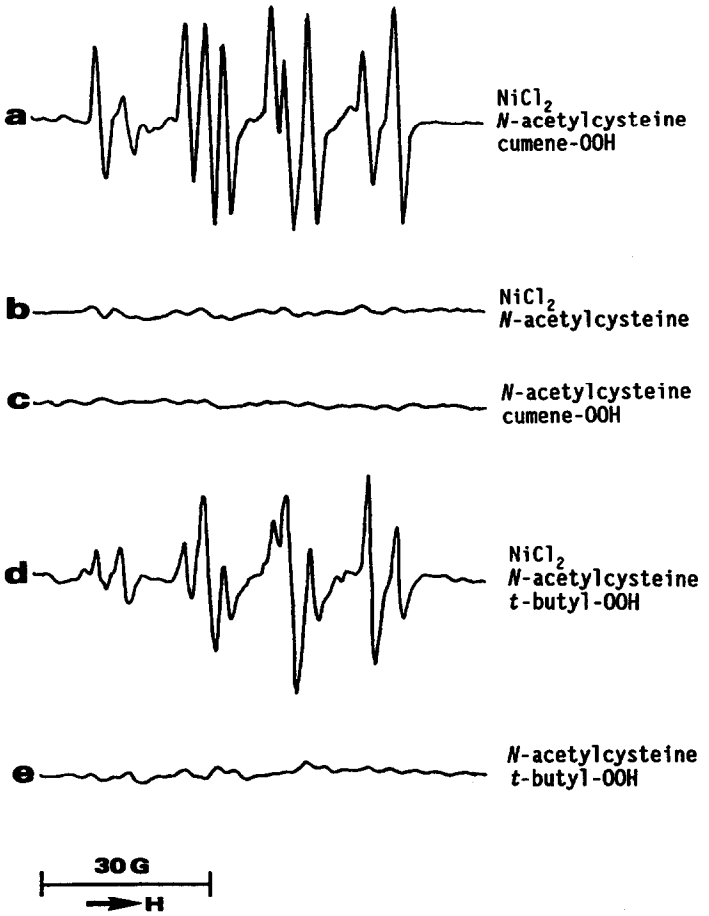

FIGURE 8. ESR spectra, recorded 6 minutes after reaction initiation, from a pH 7.2 phosphate buffer solution of 200 mM DMPO and the following reactants: (a) 1 mM $NiCl_2$, 2 mM *N*-acetylcysteine and 5 mM cumene-OOH; (b) 1 mM $NiCl_2$ and 2 mM *N*-acetylcysteine; (c) 2 mM *N*-acetylcysteine and 5 mM cumene-OOH; (d) 2 mM $NiCl_2$, 2 mM *N*-acetylcysteine and 5 mM *t*-butyl-OOH; (e) 2 mM *N*-acetylcysteine and *t*-butyl-OOH. The ESR spectrometer settings were the same as those in Figure 7.

It is known that uncomplexed Ni(II) cation is not redox active under physiologically relevant conditions [37]. However, it has been demonstrated that complexes of Ni(II) with certain oligopeptides can facilitate reaction of Ni(II) with H_2O_2 [9–12] and model hydroperoxides [21] under such conditions. Thus, it appears that chelation of Ni(II) is essential for its redox activity. The reactions may involve transient formation of Ni(III) and result in degradation of the organic ligand. Products of such reactions, including oxygen- and/or carbon-centered radicals, are capable of damaging the structure of DNA and nuclear proteins [38]. The present results provide further important examples of such redox active complexes of Ni(II) with thiol ligands, which may be formed intracellularly. As shown in this study, the Ni(II)-thiol complexes generated free radicals, including ·OH, ·R, and ·OR radicals, through interactions with molecu-

lar oxygen and/or hydroperoxides. The latter are produced in cells exposed to Ni(II) [14–21]. The mechanisms of free radical generation in these interactions might include the following: (a) Formation of ·OH radical in the reaction between Ni(II)-thiol complex and ambient oxygen. The ·OH radical then reacts with hydroperoxide to form ·R and ·OR radicals. Formation of the latter radicals from lipids and lipid hydroperoxides reacting with ·OH radical has been reported previously [39]. The ·OH radical scavengers ethanol and formate decreased the yield of ·R and ·OR radicals, supporting this conclusion. However, this result does not rule out the possibility that the reaction between the Ni(II)-thiol complex and molecular oxygen may generate a small amount of a highly oxidative species such as oxo-Ni(IV) or Ni(III) peroxide with reactivity equal to that of the ·OH radical. These species may also react with ethanol, formate, and lipid hydroperoxides to produce free radicals. Occurrence of this type of nickel species has been postulated in the Ni(II)/H_2O_2/oligopeptide systems [12]. Likewise, it was reported that oxidation by some ferrous iron species and hydrogen peroxide might be due to iron-oxo species such as ferryl ion [40, 41]. (b) Direct reaction of an Ni(II)-thiol complex with lipid hydroperoxides to generate lipid hydroperoxide-derived free radicals. The observation that the ·OH radical scavengers reduced, but did not completely suppress, the radical generation supports this conjecture. Additional support came from the data obtained using *N*-acetylcysteine. Unlike that with cysteine, the reaction mixture containing Ni(II) and *N*-acetylcysteine under aerobic conditions without hydroperoxide did not generate any detectable amount of free radicals. Addition of hydroperoxide was required to generate ·R and ·OR radicals in that mixture, albeit in low yield. A direct reaction between the Ni(II)-*N*-acetylcysteine complex and hydroperoxide seemed to be predominant. It is worthwhile to notice that under a nitrogen atmosphere, a mixture of Ni(II) and cysteine did not generate a significant amount of ·OH radical. However, when hydroperoxide was added, free radical adducts were generated, but the yield was about half that obtained under aerobic condition.

The present work also shows that reaction between thiols (except *N*-acetylcysteine) and hydroperoxides in the absence of Ni(II) does generate a small amount of free radicals. This is not surprising since it has been reported that reaction between cysteine and H_2O_2 generates ·OH and other radicals [42]. The mechanism of free radical generation from thiols and lipid hydroperoxides may be similar to that from cysteine and H_2O_2; both may be enhanced by trace amounts of transition metal ions [42]. This thiol-mediated free radical generation from lipid hydroperoxides and H_2O_2 may be partially responsible for thiol-related mutagenic effects [43].

In addition to thiols, histidine has also been reported to form a complex with Ni(II) [13, 24, 25]. Unlike with thiols, chelation of Ni(II) with histidine did not activate Ni(II) toward lipid hydroperoxides. However, the addition of histidine to a Ni(II)/cysteine/hydroperoxide mixture, in the presence of molecular oxygen, increased the yield of carbon-centered (alkyl) radicals. Part of this increase may be due to the formation of histidine-derived alkyl radicals. Since histidine was present only at 2 mM, it must have reacted with the ·OH radical site-specifically in order to compete with DMPO (200 mM). Thus, it appears that in addition to cysteine, histidine also binds to Ni(II) in close proximity to the ·OH radical-generating site. This is consistent with the formation of a ternary Ni(II)-

cysteine-histidine complex that predominates in vivo over other low-molecular weight Ni(II) carriers [25]. Site-specific reactions of this type may also occur with other cellular constituents. For example, it has been reported that 2′-deoxyguanosine is capable of forming a complex with Ni(II) [44]. Histidine enhanced oxidation of the guanine residue by H_2O_2 in that complex [13]. Perhaps cysteine alone, or in combination with histidine, could also enhance Ni(II)-mediated oxidation of guanine and other DNA bases.

It is known that most flavoenzymes contain -SH groups in their catalytic center and some of these flavoenzymes, such as glutathione reductase, function as important cellular antioxidants. Possible interactions of Ni(II) with the -SH groups of these enzymes may alter their activities toward oxidative cellular damage. Moreover, as mentioned above, in the presence of thiols, Ni(II) might be capable of generating lipid hydroperoxide-derived free radicals. These radicals can cause a variety of pathogenic reactions, including DNA damage. They have been recognized as mediators of tumor initiation and promotion [45–47]. Ni(II)-mediated lipid hydroperoxide-derived free radical formation in the presence of thiols may play an important role in the mechanism of Ni(II)-induced toxicity and carcinogenicity [21].

In conclusion, the present work demonstrates that Ni(II)-thiol complexes are capable of reacting with molecular oxygen to generate ·OH radicals. These ·OH radicals can react with other species close to their generation site, including DNA, to produce alkyl radicals. The ·OH radicals are also capable of initiating lipid peroxidation and produce alkyl and alkoxyl radicals. Coordination of Ni(II) by thiols can increase the reactivity of Ni(II) toward lipid hydroperoxides to generate lipid peroxide-derived free radicals. Thus, the interaction of Ni(II) with thiol-containing cellular constituents and related free radical generation from molecular oxygen, especially in the presence of lipid hydroperoxides, may be involved in the mechanisms of Ni(II) toxicity and carcinogenicity.

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