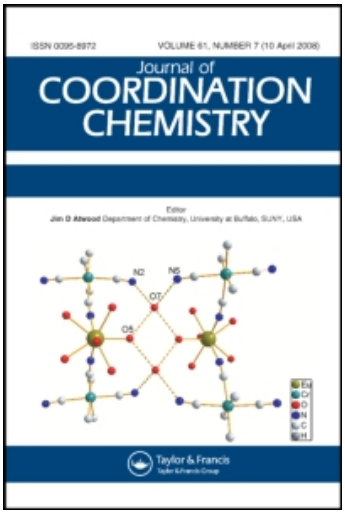

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Effect of some antioxidants on oxidative DNA damage induced by autoxidation of microquantities of sulfite in the presence of Ni(II)/Gly–Gly–L–His§

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$Ni^{II}GGH$ (GGH, glycylglycyl-L-histidine) reacts rapidly with S(IV), in air-saturated solution, to produce $Ni^{III}GGH$. A mechanism is proposed where Ni^{III} oxidizes SO_3^{2-} to $SO_3^{\bullet-}$, which reacts with dissolved oxygen to produce $SO_5^{\bullet-}$, initiating radical chain reactions. DNA strand breaks and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) formation were observed in air-saturated solutions containing micromolar concentrations of nickel(II) and S(IV). The efficacies of melatonin, (−)-epigallocatechin-gallate (from green tea), resveratrol, tannic, and ascorbic acids in terms of their inhibitory activities of DNA strand breaks and 8-oxodGuo formation were evaluated.

Keywords: DNA; Sulfite; Nickel; Gly–Gly–His; Antioxidants

1. Introduction

Human and animal exposures to S(IV) (H_2SO_3, HSO_3^-, and SO_3^{2-}) result from inhalation of SO_2, a component of industrial emissions and ingestion as preservative in food, beverages, or drugs.

The oxidation mechanism of S(IV) oxides by oxygen in the presence of iron and manganese ions is of environmental interest especially because of their relative high abundance [1, 2].

One very important study to elucidate the mechanism of this process, done in collaboration with R. van Eldik, was the oxidation of S(IV) in air-saturated solution in the presence of $Co(II)/N_3^-$ complexes which showed rapid oxidation of Co(II)

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complex and S(IV) with oxygen consumption. These processes exhibit autocatalytic behavior, that is, the oxidation reaction is catalyzed by Co(III) with formation of oxy-sulfur radicals ($SO_x^{\bullet -}$, $x = 3-5$) [3]. Under carefully selected conditions, both the oxidation and subsequent reduction of the metal ion, or vice versa, can be observed in a redox cycle demonstration experiment that exhibits two clear color changes during the redox cycling. Fascinating demonstrations of this redox cycling can be seen for Co(II)/Co(III)/N_3^- complexes and $Ni(OH)_2/Ni(OH)_3$ [4, 5].

The S(IV) induced autoxidation of Ni(II), Co(II), and Cu(II), in the presence of a complexing medium, has been studied by our research group. Detailed kinetics and mechanistic studies have been done for the systems: Mn(II)/(III)/N_3^-, Mn(II)/(III)/acetate, Co(II)/(III)/TRIS, Ni(II)/(III)/cyclam, Ni(II)/(III)/tetraglycine, Co(II)/(III)/tetraglycine, and Ni(II) and Cu(II)/(III)/tetra, penta, and hexaglycine [6–10]. Oxidation of the divalent metal ion is accelerated in the presence of S(IV) (a reducing agent) and dissolved oxygen, which may be explained by a radical mechanism. The influence of the nature of the metal ion complex was also investigated, demonstrating the importance of both the ligand and the metal ion type.

In the presence of suitable aqueous medium the formation of the metal ion in the 3+ oxidation state can be followed *via* spectrophotometric, amperometric, and luminescent measurements, which were used for the development of alternative analytical methods for S(IV) determinations in air, rain water, wine, juices, and white sugar [7, 11–13].

It has been shown that low concentrations of metal ions and complexes of cobalt, iron, chromium, manganese, nickel, and copper in the presence of S(IV) and dissolved oxygen induce DNA damage [14–18].

In this study, the DNA damage and 2′-deoxyguanosine oxidation in the presence of S(IV), Ni^{II}GGH (GGH, glycylglycyl-L-histidine), oxygen (air-saturated solution), and some antioxidants (melatonin, (−)-epigallocatechin-gallate (EGCG) (from green tea), resveratrol, tannic, and ascorbic acids) were investigated. The system S(IV)/Ni^{II}GGH/O_2 was selected since it is the first observation of DNA damage by micro quantities of S(IV) (0.5–100 μmol L^{-1}) and a Ni(II) peptide complex (0.5–200 μmol L^{-1}). It demonstrated applications to the studies on the DNA oxidation and may mimic an important metal binding [15].

Resveratrol (3,4′,5-trihydroxystilbene) and tannins are phenolic compounds naturally occurring in plants, and together with added sulfite, are present at high levels in wine. Sgambato *et al.* [19] showed that resveratrol inhibits cell proliferation and damage to DNA after exposure to oxidative agents (i.e., tobacco-smoke condensate and H_2O_2) by preventing the increase of reactive oxygen species [19].

EGCG is one of the main polyphenolic components, extracted from green tea leaves, known for its antioxidative effects.

Redox reactions of L-ascorbic acid and melatonin are of fundamental interest in chemistry, biochemistry, pharmacology, and several areas of medicine. Melatonin's efficacy in protecting against oxidative damage has been widely documented in various types of experiments conducted in animals, tissue culture, and in cell-free systems (by measuring the levels of the oxidation of the nucleobase 2′-deoxyguanosine) [20]. The efficiency of melatonin, and several metabolites of melatonin, is a result of its ability to scavenge, among others radicals, the $HO^{\bullet}$ and peroxyl radicals [20].

2. Experimental

2.1. *Reagents*

All reagents were of analytical grade. All solutions were prepared by using de ionized water purified with a Milli-Q Plus Water System (Millipore).

Melatonin, EGCG (from green tea), resveratrol, ascorbic acid, 2′-deoxyguanosine, ethidium bromide, Ficoll type 400, bromophenol blue, and others reagents used for gel electrophoresis were obtained from Sigma. Tannic acid ($C_{76}H_{52}O_{46}$), used as standard for tannins, was obtained from J.T. Baker.

Supercoiled pUC-19 DNA and electrophoresis grade agarose were purchased from MBI Fermentas.

Stock solutions of S(IV) (0.010 mol L^{-1}) were prepared daily by dissolving $Na_2S_2O_5$ (Merck) in water previously purged with nitrogen. Water was flushed with nitrogen for at least half an hour to remove dissolved oxygen. To prepare diluted solutions of S(IV), small volumes of the stock solutions were added to air-saturated water. The S(IV) content of the stock solution was standardized by iodometric titration.

Ni(II) (0.20 mol L^{-1}) stock solution was prepared from direct reaction of Ni powder (99.99%, Sigma) with doubly distilled nitric acid followed by standardization with ethylenediamine tetraacetic acid (EDTA) by a conventional procedure [21].

In the experiments, freshly prepared Ni^{II}GGH solutions were used, which were prepared by dissolving GGH in water followed by addition of Ni(II) solution (solutions were prepared to have 10% excess of peptide to prevent any $Ni(OH)_2$ precipitation). The final pH was adjusted with 0.1 mol L^{-1} NaOH or 0.1 mol L^{-1} $HClO_4$ solutions.

Air-saturated solutions were employed in all experiments for which the oxygen concentration can be considered 0.25 mmol L^{-1}. A pH meter Metrohm model 713 with a glass electrode (filled with saturate NaCl) was used in the pH measurements. The temperature was kept at 25.0°C.

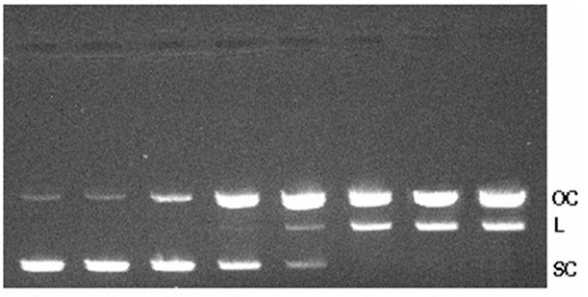
2.2. *Gel electrophoresis experiments*

The DNA strand breaks induced by the Ni^{II}GGH/S(IV)/O_2 system were determined by mixing Ni(II) complex solution with 100 ng of pUC19 plasmid DNA followed by addition of S(IV)/antioxidant in a total volume of 50 μL. The final concentrations after the mixture are indicated in figures 1–3.

Separation of the different conformations of pUC19 plasmid DNA (supercoiled, open circular; OC, and linear; L) was performed by gel electrophoresis using 0.8% agarose per 1.8 μmol L^{-1} ethidium bromide in a horizontal gel electrophoresis chamber at 30 mA for 120 min in 90 mmol L^{-1} tris-borate per 2 mmol L^{-1} EDTA buffer (pH 8.0). The bands were visualized under UV light and quantified with the ImageMaster VDS densitometer (Pharmacia Biotech, San Francisco, CA, USA).

2.3. *HPLC detection of 8-oxodGuo experiments*

The concentration of 8-oxodGuo stock solution (1.0 μmol L^{-1}) was determined by absorbance measurement at 248 nm ($\varepsilon = 12{,}900$ mol L^{-1} cm^{-1}) [22]. The calibration



S(IV) (μM)	OC (%)	L (%)	SC (%)
Lane 2: Zero	6 ± 2	0	87 ± 5
Lane 3: 2.5	22 ± 3	0	71 ± 4
Lane 4: 5.0	58 ± 3	1 ± 0.4	34 ± 2
Lane 5: 7.5	77 ± 6	5 ± 1	11 ± 1
Lane 6: 10	78 ± 4	15 ± 2	0
Lane 7: 20	72 ± 5	21 ± 2	0
Lane 8: 100	69 ± 6	24 ± 3	0

Figure 1. Formation of DNA strand breaks upon exposure of plasmid pUC19 to a 10 μmol L^{-1} Ni^{II}GGH solution in the presence of different concentrations of S(IV). [pUC19] = 5 μg mL^{-1}, pH 7.0, $T = 25.0$°C. Percentages listed are the average of three trials (error limit between 2% and 26%). DNA alone contained 7% OC (lane 1). These are air-saturated solutions.

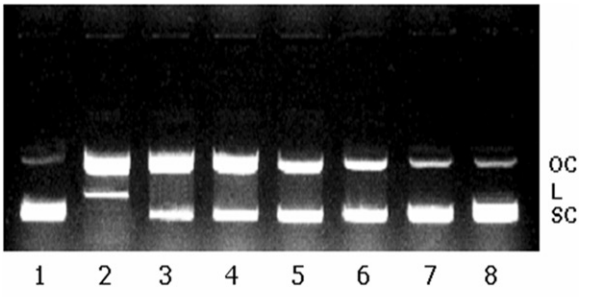
curve (peak area *vs*. concentration) was constructed using solutions in the range 0.01–0.75 μmol L^{-1} 8-oxodGuo.

In this study, 20 μL of 400 μmol L^{-1} 2′-deoxyguanosine was mixed with 20 μL of 100 μmol L^{-1} Ni^{II}GGH followed by addition of 20 μL of a mixture of 500 μmol L^{-1} S(IV)/(2.5–2500 μmol L^{-1}) antioxidant and diluted in a total volume of 100 μL (pH 7.0; 25°C). 20 μL aliquots of the sample were injected into the HPLC column. When the concentrations of 8-oxodGuo formed were out of the calibration curve range, the final solution was diluted before injection. The final concentrations after mixing are indicated in figure 4.

After mixing, the 8-oxo-7,8-dihydro-2′-deoxyguanosine formed was separated from the bulk of dGuo by HPLC and quantified by electrochemical detection [15].

3. Results and discussion

The addition of S(IV) to an air-saturated solution containing Ni^{II}GGH results in rapid formation of Ni^{III}GGH (with an absorption band at 300–400 nm).



Ascorbic acid (μM)	OC+L (%)
Lane 2: Zero	91±4
Lane 3: 0.5	61±3
Lane 4: 1.0	56±4
Lane 5: 5	41±3
Lane 6: 10	30±1
Lane 7: 50	7±0.5
Lane 8: 100	5±0.2

Figure 2. Formation of DNA strand breaks upon exposure of plasmid pUC19 to a $10\,\mu mol\,L^{-1}$ $Ni^{II}GGH$ solution in the presence of $10\,\mu mol\,L^{-1}$ S(IV) and different concentrations of ascorbic acid. $[pUC19] = 5\,\mu g\,mL^{-1}$, pH 7.0, $T = 25.0°C$. Percentages listed are the average of three trials (error limit between 2% and 26%). DNA alone contained 9% OC (lane 1) (air-saturated solutions).

Reactions 1–9 represent the main steps of the autoxidation of S(IV) in the presence of $Ni^{II}GGH$ [6–8, 15].

Initiation

$$Ni^{II}GGH + O_2 \rightarrow Ni^{III}GGH + O_2^- \quad (1)$$

Autocatalytic process

$$HSO_3^- \rightleftharpoons SO_3^{2-} + H^+ \quad pK_2 = 7.2 \quad (2)$$

$$Ni^{III}GGH + SO_3^{2-} \rightarrow Ni^{II}GGH + SO_3^{\bullet -} \quad (3)$$

$$SO_3^{\bullet -} + O_2 \rightarrow SO_5^{\bullet -} \quad (4)$$

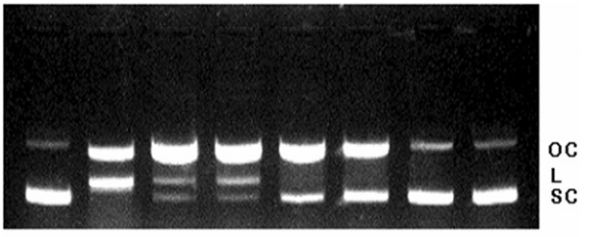
$$Ni^{II}GGH + SO_5^{\bullet -} \rightarrow Ni^{III}GGH + SO_5^{2-} \quad (5)$$

$$SO_3^{2-} + O_2 \rightarrow SO_5^{2-} \quad (6)$$

$$HSO_5^- \rightleftharpoons SO_5^{2-} + H^+ \quad pK_2 = 9.4 \quad (7)$$

$$Ni^{II}GGH + HSO_5^- \rightarrow Ni^{III}GGH + SO_4^{\bullet -} + OH^- \quad (8)$$

$$Ni^{II}GGH + SO_4^{\bullet -} \rightarrow Ni^{III}GGH + SO_4^{2-} \quad (9)$$



Melatonin (μM)	OC+L (%)
Lane 2: Zero	92±3
Lane 3: 0.5	84±2
Lane 4: 1.0	83±4
Lane 5: 5	67±3
Lane 6: 10	51±1
Lane 7: 50	7±0.7
Lane 8: 100	3±0.2

Figure 3. Formation of DNA strand breaks upon exposure of plasmid pUC19 to a $10\,\mu mol\,L^{-1}$ $Ni^{II}GGH$ solution in the presence of $10\,\mu mol\,L^{-1}$ S(IV) and different concentrations of melatonin. $[pUC19] = 5\,\mu g\,mL^{-1}$, pH 7.2, $T = 25.0^{\circ}C$. Percentages listed are the average of three trials (error limit between 2% and 26 %). DNA alone contained 8% OC (lane 1) (air-saturated solutions).

In this autocatalytic process, some Ni(III) is necessary to initiate the reactions. This species might be produced at trace level by spontaneous oxidation of $Ni^{II}GGH$ by dissolved oxygen (equation (1)). $Ni^{III}GGH$ then reacts with SO_3^{2-} to form the $SO_3^{\bullet-}$ radical (equation (3)) and further reaction with O_2 gives $SO_5^{\bullet-}$ (equation (4)). In an autocatalytic process, $Ni^{II}GGH$ (if in large excess, $2.0 \times 10^{-4}\,mol\,L^{-1}$) is oxidized to $Ni^{III}GGH$ by $SO_5^{\bullet-}$ (equation (5)). HSO_5^- and $SO_4^{\bullet-}$ can also oxidize $Ni^{II}GGH$ in subsequent steps (equations (6)–(9)). The $Ni^{III}GGH$ generated species [23] can be also reduced by SO_3^{2-} (equation (3)), continuing the chain reaction. This redox cycling depends on the critical balance between oxygen and sulfite concentrations, which will control the overall S(IV) oxidation to S(VI) and concurrent oxygen consumption.

The $SO_3^{\bullet-}$ radical formation in this reaction has also been confirmed by previous EPR spin trapping studies [15] using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). The possible involvement of $HO^{\bullet}$ in the mechanism was ruled out since the characteristic spectrum of the DMPO/$HO^{\bullet}$ adduct was not observed.

The $Ni^{III}GGH$ formation is followed by fast decomposition, which could involve the reduction of $N^{III}GGH$ by S(IV), when it is present in excess over oxygen (equation (3)).

Studies concerned with the oxidation of $Ni^{II}GGH$ by oxygen (in absence of S(IV)) assume the formation of $Ni^{III}GGH$ and Ni^{III}-superoxide complex. The loss of CO_2 from the initial Ni^{III}-superoxide complex produces the His Cα-centered free radical and peroxide with subsequent Ni^{II}Gly-Gly-α-hydroxy-D,L-histamine formation [24].

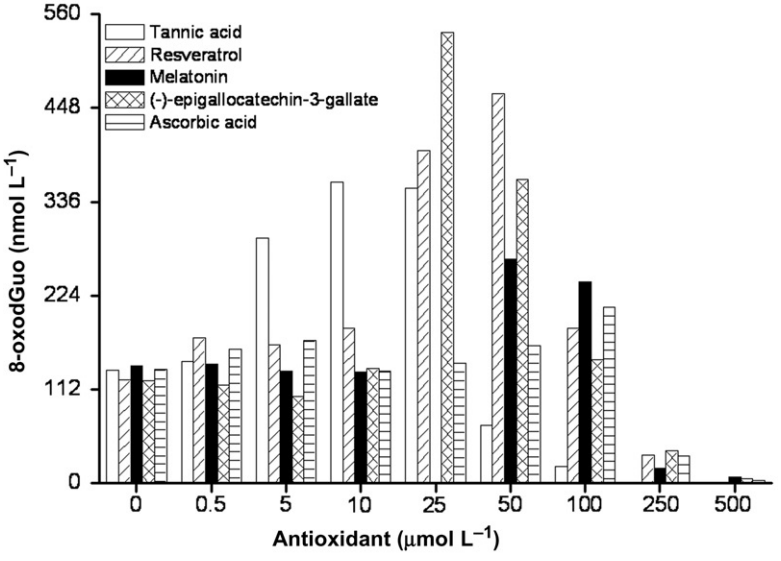


Figure 4. 8-oxodGuo formation as a function of antioxidant concentration in the presence of 20 μmol L^{-1} $Ni^{II}GGH$, 100 μmol L^{-1} S(IV), pH 7.0 (unbuffered medium), $[dGuo]_{initial} = 80\,\mu mol\,L^{-1}$; $T = 25°C$. Air-saturated solutions. $[8\text{-oxodGuo}]_{initial} = 1.7\,nmol\,L^{-1}$. The data are the average of three measurements. Error limit between 5% and 30%.

Further evidence of the formation of a highly reactive nickel superoxide complex during the $Ni^{III}GGH$ decomposition comes from our previous EPR spin trapping studies [15], with evidence for peroxy radical ($ROO^{\bullet}$) generation.

3.1. *Electrophoresis studies: DNA strand breaks by S(IV) autoxidation catalyzed by $Ni^{II}GGH$*

DNA strand break assay provides a very sensitive way to detect DNA damage. However, no information is obtained about the site specificity.

In our previous work [15], DNA strand breaks and oxidation of 2′-deoxyguanosine were observed in air-saturated solutions containing micromolar concentrations of $Ni^{II}GGH$ complex and S(IV).

In this study, pUC-19 plasmid DNA was exposed to $Ni^{II}GGH$, S(IV), and some antioxidants at different concentrations. Oxidative damage to the plasmid DNA was verified by agarose gel electrophoresis through conversion of supercoiled DNA (SC, native conformation) to OC and L forms, resulting from single- and double-strand breaks, respectively.

Quantification of DNA damage was performed by the ratio between the total amount of OC (normalized with respect to the background produced by DNA alone) or L forms produced and the total amount of DNA present.

Several control experiments were carried out in this work. No DNA damage was observed in the following conditions: S(IV) alone (20–1000 μmol L^{-1}) without and with incubation for 4 h; Ni(II) ion (added as $Ni(NO_3)_2$, 50–1000 μmol L^{-1}) in the presence or

absence of S(IV). Nevertheless, $Ni^{II}GGH$ alone only induced some damage at concentrations equal or higher than 200 μmol L^{-1}, however at lower concentrations no damage was observed [15].

Exposure of pUC19 plasmid DNA to low concentrations of $Ni^{II}GGH$ and S(IV) leads to efficient formation of DNA strand breaks. Figure 1 shows the effect of different S(IV) concentrations when $Ni^{II}GGH$ was kept constant (10 μmol L^{-1}). Increases in concentration of $Ni^{II}GGH$ complex lead to a significant enhancement of DNA strand breaks. Supercoiled DNA becomes quantitatively converted into OC and L forms when concentrations of 10–100 μmol L^{-1} S(IV) are used. The extent of the DNA damage depends on the ratio between S(IV) and $Ni^{II}GGH$ concentrations [15].

The strongly oxidant species formed in the redox cycling of $Ni^{II}/Ni^{III}GGH$ (equations (4)–(9)) may oxidize any of the four nucleosides in DNA. It is also necessary to consider that the reactive intermediates, as metallopeptide radicals and $Ni^{III}GGH\text{-}O_2^{\bullet}$, generated during the Ni(III) decomposition can interact with DNA also producing oxidative damage [24].

The reaction of $Ni^{II}GGH$ with S(IV) in air-saturated solution represents the lowest reported concentrations of S(IV) and nickel at which DNA damage has been observed [15]. These findings are particularly relevant in the studies of S(IV) and nickel toxicity. In addition, these studies showed oxidative DNA damage using S(IV), a reducing agent, rather than highly reactive oxidants, such as HSO_5^- [25].

Under these conditions (figure 1), the experiments were carried out by addition of ascorbic acid (figure 2) or melatonin (figure 3) by keeping the S(IV) concentration constant (10 μmol L^{-1}).

The increase of melatonin and ascorbic acid concentrations decreased the DNA strand breaks. At lower concentrations (0.5–10 μmol L^{-1}) ascorbic acid had higher effect than melatonin on decreasing the DNA strand breaks (figures 2 and 3, lanes 3–6).

3.2. *Oxidation of 2′-deoxyguanosine by S(IV) autoxidation catalyzed by $Ni^{II}GGH$*

2′-deoxyguanosine (dGuo) is the nucleobase usually used to verify oxidative damage in DNA; among all the nucleobases it is the most susceptible to oxidation [26]. This dGuo can be oxidized to its principal product, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), the most common oxidative lesion observed in DNA, in the presence of strong oxidizing agents, as those generated during S(IV) autoxidation catalyzed by $Ni^{II}GGH$. With HPLC it is possible to detect and quantify 8-oxodGuo.

In this study, some control experiments were also carried out. After injection of a solution containing only 80 μmol L^{-1} dGuo a small peak attributable to 8-oxodGuo was observed, indicating that the reagent dGuo contains $[\text{8-oxodGuo}]_{\text{initial}} = 1.7$ nmol L^{-1}.

When a mixture of dGuo plus S(IV) or dGuo plus $Ni^{II}GGH$ was injected, no changes in the intensity of the peak occurred. However, in an air-saturated solution containing dGuo, $Ni^{II}GGH$ and S(IV), a considerable increase in the intensity of the peak was observed. This indicated generation of high levels of 8-oxodGuo and that the products of the S(IV) autoxidation are able to oxidize dGuo to 8-oxodGuo and possibly can also oxidize guanine in DNA [15].

In this work, studies were done to compare the relative efficacies of tannic and ascorbic acids, melatonin, (−)-EGCG, and resveratrol as antioxidants on the oxidation of dGuo in the presence of $Ni^{II}GGH$, S(IV) and dissolved oxygen.

Ascorbic acid was an efficient antioxidant at concentrations greater than $100\,\mu mol\,L^{-1}$ (figure 4). The results from the electrophoresis experiments on DNA (figure 2) carried out at a lower S(IV) concentration ($10\,\mu mol\,L^{-1}$) showed that the %(OC + L) gradually decreases with increasing ascorbic acid concentration and is completely inhibited at higher concentrations ($100\,\mu mol\,L^{-1}$ ascorbic acid).

In figure 4, melatonin behaved as an antioxidant at concentration greater than $100\,\mu mol\,L^{-1}$. The levels of 8-oxodGuo were very low for $250\,\mu mol\,L^{-1}$ melatonin, with 94% reduction for $500\,\mu mol\,L^{-1}$ melatonin. In the range of 50–$100\,\mu mol\,L^{-1}$ the estimated errors are 40%; however, melatonin appears to have some pro-oxidant effect. The results from electrophoresis experiments (figure 3) showed that melatonin gradually decreases the DNA damage produced in the presence of $Ni^{II}GGH/S(IV)/O_2$ (figure 3, lane 2: $S(IV) = 10\,\mu mol\,L^{-1}$) by inhibiting formation of OC and L. Complete inhibition is obtained at $100\,\mu mol\,L^{-1}$ melatonin.

Tannins are a heterogeneous mixture of natural polymeric phenolic compounds obtained from vegetables with a high molecular weight. Due to the phenolic groups, tannin precipitates proteins and forms complexes with metal ions.

Tannic acid had a pro-oxidant effect at lower concentrations of 5–$25\,\mu mol\,L^{-1}$ (figure 4). However, when concentrations higher than $50\,\mu mol\,L^{-1}$ were used, an 85% decrease in the formation of 8-oxodGuo was observed.

EGCG had a similar pro-oxidant effect in the concentration range 25–$50\,\mu mol\,L^{-1}$. However, at concentration equal to $250\,\mu mol\,L^{-1}$, EGCG behaved as an antioxidant.

Resveratrol had almost no effect when present at 0.5–$10\,\mu mol\,L^{-1}$, with pro-oxidant behavior at 25–$50\,\mu mol\,L^{-1}$ (data with high error). Concentrations higher than $250\,\mu mol\,L^{-1}$ reduced the formation of 8-oxodGuo to approximately 70% (figure 4).

Guanine is the most susceptible to oxidative damage ($E^{o}Guo^{\bullet}/Guo = 1.29\,V$ *vs.* NHE for guanosine, pH 7) [26]. In the presence of $Ni^{II}GGH$ and S(IV), dGuo is converted into the product 8-oxodGuo, showing that guanine oxidation can occur in DNA (figure 4).

$SO_3^{\bullet-}$ radical ($E^{o}SO_3^{\bullet-}/SO_3^{2-} = 0.76$ *vs.* NHE, pH > 7) [27], initially formed (equation (3)), and $SO_5^{\bullet-}$ ($E^{o}SO_5^{\bullet-}/HSO_5^{-} = 1.10$ *vs.* NHE, pH 7) [27] are unlikely to oxidize guanine because of their low redox potentials; therefore, other species must be involved in DNA damage. HSO_5^{-} ($E^{o}HSO_5^{-}/SO_4^{2-} = 1.75$ *vs.* NHE, pH 3–9) [27] and $SO_4^{\bullet-}$ radical ($E^{o}SO_4^{\bullet-}/SO_4^{2-} = 2.43$–$3.08$ *vs.* NHE) [27] could easily oxidize not only guanine but also other nucleosides.

At this point, it is still difficult to unambiguously identify the oxidative intermediates, generated in the S(IV) autoxidation catalyzed by metal ion complexes that are responsible for DNA damage.

The relative efficacies of theses antioxidants, which have widely different molecular structures and reactive groups, on preventing DNA damage and 8-oxodGuo formation, will depend on the concentration ratio of [S(IV)]:[antioxidant]. Direct comparison of the data from electrophoresis and HPLC experiments is difficult because of the different sensitivity of the techniques used. The mechanism of DNA damage must be different from 2′-deoxyguanosine oxidation and could not be elucidated using these techniques.

4. Conclusion

Except for tannic acid, all other antioxidants used, figure 4, had a marked effect of reducing the formation of 8-oxodGuo only at concentrations higher than 100 μmol L^{-1} (the same as used for S(IV)), inhibiting completely the oxidation of dGuo due to radical formation in the system $Ni^{II}GGH/S(IV)/O_2$.

Studies of DNA damage in the presence of oxidants ($KHSO_5$ (oxone), H_2O_2 and a mixture H_2O_2/ascorbate) and Ni(II) complexes (tetraazamacrocycles) showed that some nickel complexes alone are excellent promoters of oxidative DNA modifications. The intrinsic reactivity of Ni(II) complexes was related to the availability of vacant coordination sites through a square-planar geometry, the overall positive charge on the complex and relatively high reduction potential of Ni(III)/Ni(II) [28].

Ni(II) forms a square-plane with the tripeptide GGH, by coordination of the terminal amino nitrogen, two deprotonated amide nitrogens, and the imidazole–N3 donors [24, 29]. Potentiometric studies showed that at pH higher than 6.4 the major species present in solution is an anionic complex [24, 29].

Studies from Kasprzak and Hernandez [30] showed that after incubation (37°C for 5–9 days) both Ni_3S_2 and Ni(II) increased formation of 8-dihydro-2′-deoxyguanosine from 2′-deoxyguanosine exposed to H_2O_2/ascorbate. However, Ni(II), unlike Ni_3S_2, increased the extent of dGuo deglycosylation when added to the dGuo + H_2O_2/ascorbate.

The role of ascorbate in nickel toxicity and carcinogenesis has been studied by several authors [28, 31–35]. Spin-trapping experiments showed that nickel(II) in the presence of L-ascorbic acid is not a Fenton active metal and no reactive organic species are generated even with added oxidants (i.e., H_2O_2) [32]. It was suggested that carbon-centered radicals derived from fragmentation of L-ascorbic acid may induce single- and double-strand scissions to DNA.

As reported in the literature many antioxidants can act as pro-oxidants depending on the presence of some metal ion. Cu(II) by itself rarely cleaves DNA. However, EGCG [36, 37] and resveratrol [38, 39] complex with Cu(II) and generate radicals [37] or copper–peroxide complex [38] and cleave DNA. Similar studies showed that EGCG in the presence of Ag(I) have repressive effect, while Ni(II) showed little [37].

DNA oxidatively damaged by Cr(III) and H_2O_2 is markedly reduced by antioxidants, such as melatonin (and related molecules), resveratrol, and uric acid [20, 40].

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