Nickel (II) as a temporary catalyst for hydroxyl radical generation

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Many in vivo studies show peroxidative damage during nickel toxicity, suggesting the generation of oxygen-activated species. Using the murexide (5,5'-nitrilodibarbituric acid ammonium salt) bleaching technique, we attempted to spectroscopically determine whether there are any histidyl-peptides-Ni (II) complexes able to catalyze a nickel-dependent reduction of hydrogen peroxide leading to free oxygen radical production. We show that peptides containing the glycyl-glycyl-L-histidyl sequence trigger nickel-dependent production of oxygen radicals which can damage proteins, cause a rapid loss of tryptophan and a significant production of bityrosine and also induce peroxidation of polyunsaturated fatty acids. During the reaction, the histidine residue in the peptide is selectively damaged and breakdown of the peptide switches off hydroxyl-radical production.

Lipid peroxidation; Fenton reaction; Nickel (II); Oxygen-free radical; Protein

1. INTRODUCTION

Iron, a dietary trace element, has received a great deal of attention [1]. Changes in its absorption indirectly affect the balance of cobalt and nickel, which also belong to the 8th subgroup in the periodical system of elements and which share at least part of the iron absorptive mechanism [2].

In vivo, nickel like iron was reported to increase lipid peroxidative damage [3,4]. The peroxidative potential of iron is generally related to its ability to generate, in situ, reactive oxygen species through reactions commonly referred to as Haber-Weiss and Fenton chemistry [5] and summarized as follows:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + HO^0$$

Direct catalysis of such a reaction by nickel has not yet been demonstrated. In vivo, the carcinogenic and toxic activities of nickel are thought to result in depletion of glutathione peroxidase [4]: increased levels of reduced glutathione may indirectly help to generate free radicals by reducing ferric ions to ferrous ions. On the contrary, in spin trapping EPR studies of isolated systems, Inoue and Kawanishi [6] have reported the formation of significant amounts of hydroxyl radical adducts and superoxide adducts with glycylglycyl-L-histidine, triglycine, tetraglycine and pentaglycine but not with glycyl-L-histidine or glycylglycine. These authors studied the conditions under which activated oxygen species could be produced by the reaction of Ni(II) oligopeptides with hydrogen peroxide but, as they point out, spin trapping may detect both free and nickel peptide-bound hydroxyl radicals. Moreover, Pou et al. [7] have shown that spin traps are susceptible to metal ion-catalyzed air oxidation. We tried to determine the possible production of free activated oxygen species mediated by nickel by using radical sources which included hydrogen peroxide, nickel chloride and some histidylpeptides and tested them with murexide.

As previously reported [8] oxygen free radical production can be easily detected by decreases in the maximum absorbance of murexide solutions (between 460–520 nm); we compared the behaviour of murexide solutions containing hydrogen peroxide and either Cu(II) or Ni(II) when histidyl peptides were added.

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2. MATERIALS AND METHODS

2.1. *Protein damage*

The loss of tryptophan in bovine serum albumin and lactate dehydrogenase was measured by the fluorescence emission decreases (relative to the number of tryptophan residues and the protein conformation) at 345 nm (excitation at 290 nm) with a Perkin Elmer MPF3 spectrofluorometer at pH 7.5, when 10^{-2} M hydrogen peroxide and 100 μM $NiCl_2$ were added to the protein solutions (0.33 mg/ml), with (——) or without (– – –) 100 μM glycylglycyl-L-histidine (Fig. 1). The production of bityrosine by bovine serum albumin and lactate dehydrogenase was measured by the same method described above for tryptophan loss, except the emission and excitation wavelengths were at 400 nm and 325 nm, respectively. Since bityrosine can be formed by direct tyrosine oxidation with hydrogen peroxide, relative fluorescence values indicate the difference between the observed fluorescence intensities with and without the peptide.

2.2. *UV absorption spectra of arachidonic acid oxygenated derivatives*

300 μl solution containing 3.2 mM arachidonic acid, 10 mM hydrogen peroxide in 0.02 M phosphate buffer, pH 8 was incubated for 1 h at 25°C in the presence of 3.3 mM Ni(II), 6.6 mM glycylglycyl-L-histidine or 3.3 mM Cu(II). 50 μl was extracted twice

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with hexane (1 ml), dried over Na_2SO_4 and evaporated under vacuum. The residue was diluted with hexane (300 μl) and then its absorption spectrum recorded with a Cary 118C spectrometer using 1 mm pathlength cuvettes.

2.3. *Bleaching of murexide by organic hydroperoxides*

The decrease in the absorbance of a solution containing 70 μM murexide, 100 μM Ni(II), 200 μM glycylglycyl-L-histidine was measured as a function of time by means of a Cary 118C spectrometer at 470 nm, after addition of 70 mM *t*-butylhydroperoxide or 25 mM cumene hydroperoxide and expressed as nanomoles of murexide bleached per min.

2.4. *Amino acid composition*

Samples were hydrolyzed under vacuum at 110°C for 24 h in constant boiling HCl. The hydrolysates were analyzed with a Waters Picotag system (Waters Associates, Milford, MA).

3. RESULTS

Murexide bleaching was observed in the presence of copper, only when no peptide was present, otherwise peptide-Cu(II) complexes were formed (characterized by weak absorptions [9] of 600–630 nm) which prevented the formation of oxygen free radicals.

On the contrary, no murexide bleaching was observed with free Ni(II) ions and murexide bleaching occurred only in solutions containing peptides with the glycylglycyl-L-histidyl sequence (Table I). When these solutions were incubated with hydroxyl-radical scavengers (ethanol, dimethylsulfoxide and butanol) no bleaching was observed, indicating that glycylglycyl-L-histidine-Ni(II) probably reacted with hydrogen peroxide to produce oxygen free radicals in vitro.

We examined the ability of this system to damage proteins in bovine serum albumin and lactate dehydrogenase. Fig. 1 shows that in the presence of hydrogen peroxide, glycylglycyl-L-histidine-Ni(II) produced protein modifications, tryptophan loss and bityrosine production [10], also observed when proteins

Table I

Oxidation rates of murexide in the presence of hydrogen peroxide, nickel ions and histidyl-peptides (the experimental conditions are those used to measure the bleaching of murexide by organic peroxides)

Nickel chelator	Murexide bleaching (nmol/min)
None	0
Glycylglycyl-L-histidine	10.5
Glycylglycyl-L-histidyl-glycine	8.5
L-Alanyl-glycyl-glycyl-L-histidine	2.0
Glycylglycyl-L-histidyl-L-alanine	5.5
Glycyl-L-phenylalanine	0
Glycylglycine	0

Negative results were obtained with the following peptides: glycyl-L-histidine, L-histidylglycine, L-carnosine, L-anserine, glycyl-L-histidylglycine, L-histidylglycyl-L-lysine, glycyl-L-histidyl-L-lysine.

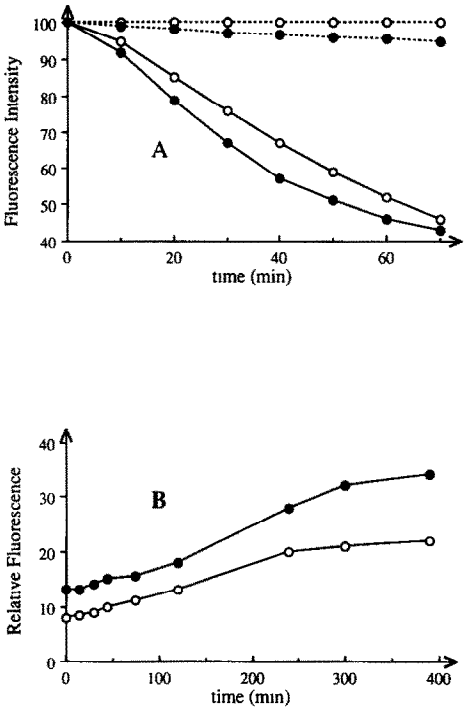

Fig. 1. Protein damage following exposure to the Ni(II)/glycylglycyl-L-histidine/hydrogen peroxide system: (A) loss of tryptophan, and (B) production of bityrosine (bovine serum albumin (○), lactate dehydrogenase (●) with (——) and without (– – –) glycylglycyl-L-histidine).

were exposed to oxygen radicals produced by water radiolysis [11]. Tryptophan destruction depends on the degree of solvent exposure of residues on the outside of the protein. Thus, loss of tryptophan may indicate protein degradation. Bityrosine, which is a covalently bound biphenol produced by reaction between tyrosyl radicals, may be more likely to be formed between two protein molecules than within single proteins. Bityrosine formation may thus indicate protein aggregation.

Fatty acid peroxidation is a sensitive marker of cellular membrane injury involving reactive oxygen species. Thus, we tested the effect of free and complexed Ni(II) ions on the peroxidation of arachidonic acid in the presence of hydrogen peroxide. Fig. 2 shows the appearance of an absorption maximum at 230–234 nm with a shoulder in the 260–280 nm region due to the formation of conjugated dienes when arachidonic acid was subjected to complexed Ni(II) ions. Fatty acid peroxidation was confirmed by the TBA test [12].

Similar results were obtained with the following peptides: glycylglycylglycyl-L-histidine, L-alanylglycylglycyl-L-histidine, glycylglycyl-L-histidineglycyl.

A Fenton type reaction of Ni(II) peptide complex with hydrogen peroxide would be the simplest explanation for these results.

When organic hydroperoxides such as *t*-butylhydroperoxide or cumene hydroperoxide were

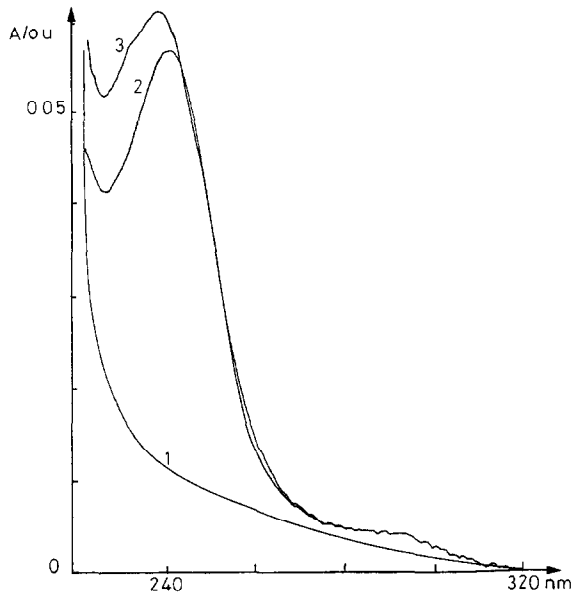

Fig. 2. UV absorption spectra of arachidonic acid subjected to peroxidation by hydrogen peroxide alone (1) and in the presence of Ni(II)/glycylglycyl-L-histidine complex (2) or Cu(II) ions (3).

substituted for hydrogen peroxide, murexide bleaching was also observed in the presence of glycylglycyl-L-histidine-Ni(II) complex. This corresponded to a hydroxyl radical production and showed that Ni(II) ions, when complexed with glycylglycyl-L-histidyl-peptides, can also disproportionate fatty acid hydroperoxides (Table II).

Table II

Bleaching of murexide by organic hydroperoxides in the presence of Ni(II)/glycylglycyl-L-histidine

	Murexide bleaching (nmol/min)
None	0
Hydroxy peroxide	10.5
t-Butylhydroperoxide	5.6
Cumene hydroperoxide	4.9

Table III

Time dependent changes in the amino acid composition of glycylglycyl-L-histidine through reaction with Ni(II)/hydrogen peroxide

	Molar ratio (%)	
	0 h	24 h
Asp	0	4.93
Gly	65.3	65.22
His	33.3	10.57
NH_4OH	1.4	19.28

Molar ratios represent the mole concentration of each amino acid per total amino acids.

Moreover, we observed a time-dependent loss of the peptide itself through a reaction with Ni(II)/H_2O_2 (Table III). The histidine residue in the peptide was selectively damaged while the glycine residues remained almost intact; also trace amounts of aspartate were detected accompanying the selective loss of histidine. The considerable production of ammonia during histidine damage might come from the breakdown of the imidazole ring leading to formation of acidic amino acids.

4. DISCUSSION

Several scientists have argued that in vivo chelation of metal ions prevents most of the 'metal-catalyzed Haber-Weiss reaction'. Conversely, our results show that some biological chelators can temporarily trigger the production of hydroxyl radicals which switch off when chelators are broken down by the generated radicals.

As reported by Athar et al. [4], Ni(II) ions could trigger peroxidation damage of lipids by lowering glutathione peroxidase activity. Moreover, our results show that, when chelated with peptides containing the glycylglycyl-L-histidine sequence, Ni(II) ions could also peroxidize lipids either through hydrogen peroxide disproportionation and hydroxyl radical production or directly by reaction with the lipid hydroperoxides.

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