SOD-like activity of complexes of nickel(II) ion with some biologically important peptides and their novel reactions with hydrogen peroxide

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(Received February 12, 1993; revised June 18, 1993)

Abstract

At physiological pH values, nickel(II) complexes of oligopeptides containing histidine in the third position can quench superoxide ion (O_2^-) more easily than other Ni(II)–oligopeptide complexes. Furthermore, it is evidenced that Ni(II) complexes of oligopeptides containing histidine in the third position can catalyze the disproportionation of hydrogen peroxide (H_2O_2) to yield O_2^-, detected by electron spin resonance (ESR) spin trapping and nitro blue tetrazolium (NBT) methods.

Introduction

Of the toxicological effects of nickel, carcinogenesis has been most intensively studied [1–4]. The epidemiological evidence is unequivocal for excess risk of respiratory cancer among nickel refinery workers and animal studies have confirmed the carcinogenic potential of crystalline, relatively water-insoluble nickel compounds. Exposure to mineral dusts induce, in tissues, the activation of alveolar phagocytic cells, a process involving the release of reduced oxygen species, i.e. superoxide ion (O_2^-) and hydrogen peroxide (H_2O_2), whose interactions with Ni(II) oligopeptides may yield hydroxyl radicals or oxene moiety $(NiO)^{2+}$ with subsequent lesions of DNA [1].

In order to ascertain the possibility releasing the active oxygen species, the redox chemistry of Ni(II) complexes with some oligopeptides in the presence of O_2^- or H_2O_2 has been studied by electron spin resonance (ESR) spin trapping and nitro blue tetrazolium (NBT) methods. Eight oligopeptides have been chosen: GlyGlyGlyGly; HisGlyGly (a peptide containing histidine at the NH_2 terminal); carnosine and GlyHisGly (peptides containing histidine in the second position); GlyGlyHis, GlyGlyHisGly, GlyHisHisGly(L,L), and GlyHisHisGly(D,L) (peptides containing histidine in the third position).

It is well known that the Ni(II) ion forms more interesting complexes with peptides than other metal ions. The Ni(II)–oligopeptide complexes adopt geometries from hexacoordinate (octahedral) exhibiting two weak absorption bands in the visible region near 380 and 620 nm to planar giving rise to yellow complexes exhibiting an absorption band in the 410–450 nm region [5]. However, the correlation between the coordination structures of the Ni(II) complexes and their reactivities towards O_2^- and H_2O_2 has not been yet studied. In this paper we report that the planar, yellow Ni(II) complexes strongly quench O_2^- and further disproportionate H_2O_2 to yield O_2^-.

Experimental

Materials

Carnosine, GlyGlyGlyGly and GlyGlyHis were purchased from Sigma Chemical Co. Other peptides were synthesized by classical peptide chemistry in solution. NBT was purchased from Dojin Kagaku Co. Hypoxanthine (HPX) was purchased from Sigma Chemical Co. Superoxide dismutase (SOD) and xanthine oxidase (XO) were purchased from Boehringer. 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) was purchased from LABOTECK and used without further purification.

Procedure

Stock aqueous solutions of Ni(II) peptides (1 mM) were prepared by addition of 10% excess peptide to

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$NiCl_2$ (Wako Pure Chemical Co.). The concentration of H_2O_2 in aqueous solutions was determined by titration with 0.10 M $KMnO_4$. Deionized and triply distilled water was used throughout. O_2^- was generated by the HPX (0.5 mM)/XO (0.1 U/ml) system at pH 7.4 in 0.10 M phosphate buffer.

ESR measurements

The production of O_2^- was followed by spin trapping using DMPO (125 mM) as a spin trap. ESR spectra were recorded at 1 min after the addition of XO to the mixing solutions of HPX and DMPO. The reactions of Ni(II)–oligopeptide complexes with H_2O_2 were performed in 0.10 M phosphate buffer at pH 7.4 by mixing 0.25 mM Ni(II) oligopeptides with H_2O_2 (25 mM) in the presence of DMPO (25 mM) [6]. In these experiments, ESR spectra were recorded at 1 min after addition of Ni(II) complexes to the mixture of H_2O_2 and DMPO. ESR measurements were carried out on a JEOL JES-RE-1X ESR spectrometer (X-band) with 100 kHz field modulation. ESR spectra were recorded at room temperature in a JEOL flat quartz cell. ESR parameters were calibrated by comparison with standard Mn^{2+}/MgO marker. In addition, the magnetic field was calibrated with an NMR fieldmeter (JEOL ES-FC5).

Determination of O_2^- with NBT method

Reactions were started by addition of H_2O_2 (25 mM) to the mixture of Ni(II) complexes (0.25 mM) and NBT (0.0125 mM) in 0.10 M phosphate buffer at pH 7.4 and were followed by the increase of the absorbance at 560 nm due to blue formazan [7]. Visible absorption spectra were recorded on a Hitachi U-3210 spectrophotometer at room temperature.

Results

Interactions of Ni(II)–oligopeptide complexes with superoxide ion

It has been established that the HPX/XO system induces a high concentration of O_2^- at pH 7.4. This system gave, in the presence of DMPO, a prominent signal of DMPO–O_2^- consisting of twelve lines (a^N (1) = 1.43 mT, a^H (1) = 1.15 mT, a^H (1) = 0.13 mT) (Fig. 1). When SOD (3000 U/ml) was added to this O_2^--generating system, the ESR spectrum due to DMPO–O_2^- entirely disappeared. This fact indicates that O_2^- is quenched by SOD. The efficiencies of Ni(II)–oligopeptide complexes in quenching O_2^- were investigated according to this quenching method. The results obtained are summarized in Table 1. In the Ni(II)–carnosine, –HisGlyGly, –GlyHisGly and –GlyGlyGlyGly complexes formation of the DMPO–O_2^- adduct was depressed slightly. Formation of the DMPO–O_2^- adduct was, however, much more suppressed by the Ni(II)–GlyGlyHis, –GlyGlyHisGly, –GlyHisHisGly(L,L) and –GlyHisHisGly(D,L) complexes. These results indicate that the four Ni(II)–oligopeptides containing a histidyl residue in the third position reveal a strong SOD activity, and the other four Ni(II)–oligopeptide complexes reveal a weak SOD activity.

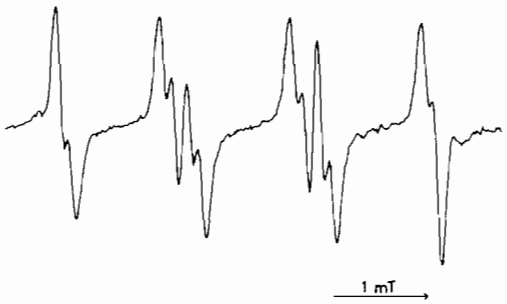


Fig. 1 ESR spectrum observed from the hypoxanthine–xanthine oxidase system in the presence of DMPO at pH 7.4. Conditions: HPX, 0.5 mM; XO, 0.1 U/ml, DMPO, 125 mM. Instrument settings: microwave power, 10 mW; amplitude, 4 × 100, modulation amplitude, 0.079 mT; time constant, 0.03 s; scan time, 2 min

TABLE 1. Dismutation of superoxide by Ni(II)–peptide complexes

Ni(II) complexes	Inhibition of O_2^- [a] (%)
Ni(II)–carnosine	27.6
Ni(II)–HisGlyGly	21 6
Ni(II)–GlyHisGly	5 1
Ni(II)–GlyGlyHis	80.9
Ni(II)–GlyGlyGlyGly	3 7
Ni(II)–GlyGlyHisGly	57 7
Ni(II)–GlyHisHisGly(L,L)	63 7
Ni(II)–GlyHisHisGly(D,L)	70 1

[a]Percent inhibition was expressed as follows:
% = ESR signal intensity of DMPO–O_2^- in the presence of Ni(II)/ESR signal intensity of DMPO–O_2^- in the absence of Ni(II) × 100.

Interactions of Ni(II)–oligopeptide complexes with hydrogen peroxide

It is thought that the hydroxyl radical ($^{\cdot}OH$) is the most deleteriously active oxygen species against living organisms [8]. This radical is chemically generated by the metal-dependent reduction of hydrogen peroxide. Usually, the Fe(II) ion is used as the metal ion. This reaction is called the Fenton reaction. The reactivities of Ni(II)–oligopeptide complexes with H_2O_2 were investigated. The results obtained are shown in Table 2. In the Ni(II)–GlyGlyHis, –GlyGlyHisGly, –GlyHisHisGly(L,L) and –GlyHisHisGly(D,L) complexes, an ESR spectrum similar to that of the DMPO–O_2^- adduct (Fig. 1) was observed. This signal disappeared when SOD was added to the Ni(II)–oligopeptide com-

TABLE 2. Reactions of Ni(II)–peptide complexes with hydrogen peroxide

Ni(II) complexes	ESR signal intensity	NBT abs./min ($\times 10^{-3}$)	λ_{max} (nm) (ϵ (M^{-1} cm^{-1}))
Ni(II)–GlyGlyHis	0.52	51	425(121)
Ni(II)–GlyGlyHisGly	0.35	20	425(129)
Ni(II)–GlyHisHisGly(L,L)	0 19	2	425(121)
Ni(II)–GlyHisHisGly(D,L)	0 65	64	423(133)
Ni(II)–carnosine	0 00	0	398(9)
Ni(II)–HisGlyGly	0.00	0	381(9)
Ni(II)–GlyHisGly	0.00	0	584(8)
Ni(II)–GlyGlyGlyGly	0 00	0	394(11)

plexes–H_2O_2 system. On the other hand, the Ni(II)–carnosine, –HisGlyGly, –GlyHisGly and –GlyGlyGlyGly complexes exhibited a scarcely detectable ESR signal due to the DMPO–O_2^- adduct. From these results, it is suggested that O_2^- was produced during the reaction of H_2O_2 with Ni(II) complexes of oligopeptides containing a histidyl residue in the third position. In order to ascertain this suggestion, we have further examined the reactions of H_2O_2 with Ni(II)–oligopeptide complexes using the NBT method. It has been established that NBT is a useful reagent for the determination of O_2^- [9]. NBT is easily reduced by O_2^- to give the blue formazan which has an absorption maximum at 560 nm. Thus the increase of absorbance at 560 nm was followed after mixing the Ni(II)–oligopeptide complexes with H_2O_2. As shown in Table 2, Ni(II) complexes with oligopeptide containing a histidyl residue in the third position show an increase of absorbance at 560 nm. This result confirmed the suggestion that O_2^- may be formed during the reactions of Ni(II)–oligopeptide complexes such as Ni(II)–GlyGlyHis, –GlyGlyHisGly, –GlyHisHisGly(L,L) and –GlyHisHisGly(D,L) with H_2O_2. The generation of O_2^- may be explained by the following schemes, similarly to the dismutation of H_2O_2 by catalase and peroxidase [1].

$$Ni(II) + H_2O_2 \rightleftharpoons (NiO)^{2+} + H_2O \quad (1)$$

$$(NiO)^{2+} + H_2O_2 \rightleftharpoons Ni(III) + O_2^- + H_2O \quad (2)$$

In these schemes, the oxene species, $(NiO)^{2+}$, is postulated as an intermediate.

Spectral measurements of Ni(II)–oligopeptide complexes

Absorption maxima of the visible region due to d–d transitions in the central Ni(II) ion of Ni(II)–oligopeptide complexes are shown in Table 2. It is apparent that Ni(II) complexes of oligopeptides containing a histidyl residue in the third position exhibit a maximum around 420 nm. These facts suggest that these complexes have a square planar coordination [5]. On the other hand, other peptide complexes of the Ni(II) ion exhibit a maximum around 380 nm, suggesting these complexes have an octahedral coordination [5].

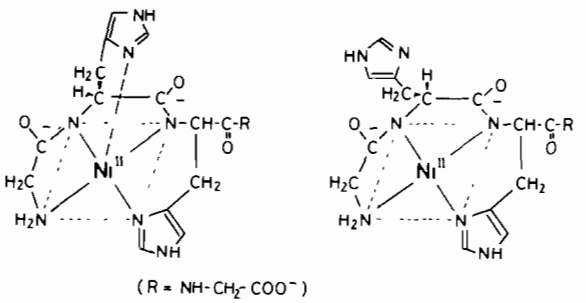
Discussion

The reactivities of some Ni(II)–oligopeptide complexes towards O_2^- and H_2O_2 have been investigated by the ESR spin trapping method. O_2^- was remarkably quenched by Ni(II) complexes of oligopeptides containing a histidyl residue in the third position, such as Ni(II)–GlyGlyHis, –GlyGlyHisGly, –GlyHisHisGly(L,L) and –GlyHisHisGly(D,L), as shown in Table 1.

Ni(II)–carnosine, –HisGlyGly, –GlyHisGly, –GlyGlyGlyGly, –GlyGlyHis, –GlyGlyHisGly, –GlyHisHisGly(L,L) and –GlyHisHisGly(D,L) reacted with H_2O_2 differently as shown in Table 2. Formation of O_2^- was observed during the reactions of Ni(II)–GlyGlyHis, –GlyGlyHisGly, –GlyHisHisGly(L,L) and –GlyHisHisGly(D,L) with H_2O_2. From these results, it is assumed that the square-planar Ni(II) complexes of oligopeptides containing a histidyl residue in the third position can dismutate H_2O_2 to yield O_2^-, but the octahedral Ni(II) complexes with other peptides cannot dismutate H_2O_2 under the same conditions. The nickel–peptide complexes exhibiting a redox potential from 0.96 to 0.79 V in the $Ni^{II, III}$–peptide couples are expected to be square-planar in the divalent state and tetragonal in the trivalent state [10]. Ni(II)–GlyGlyHis and –GlyGlyHisGly are complexes of this type [10]. Therefore, it is thought that the Ni(II)–oligopeptide complexes of which the higher oxidation state is more stable can dismutate H_2O_2.

On the other hand, it is of interest that the reactivities of the diastereoisomeric pair of Ni(II)–GlyHisHisGly towards H_2O_2 differ greatly from each other as shown in Table 2. The almost same absorption maxima of the diasteroisomeric pair of Ni(II)–GlyHisHisGly complexes, as shown in Table 2, suggests that both complexes have the same coordination structure in which the peptide chelates to the Ni(II) ion as a quadridentate

ligand with four nitrogen donor atoms [11]. Therefore, the different reactivity may be caused by the enantiomer of the histidyl residue in the second position of GlyHisHisGly. This suggests that stability in the $(NiO)^{2+}$ or Ni(III) state of the diasteroisomeric pair of the Ni(II)–GlyHisHisGly complex is affected by the enantiomer of the histidyl residue in the second position. The space-filling molecular models (Corey-Pauling-Koltun atomic models) suggest that the imidazole rings of the histidyl residue in the second position lie at the axial position of the Ni(II) plane in Ni(II)–GlyHisHisGly(D,L) and at the equatorial position of the Ni(II) plane in Ni(II)–GlyHisHisGly(L,L) as shown in Fig. 2. The enhanced activity of Ni(II)–GlyHisHisGly(D,L) compared to Ni(II)–GlyHisHisGly(L,L) may be brought about by the stabilization of the higher oxidation of the $(NiO)^{2+}$ species due to the axial imidazole donor. A similar phenomenon suggesting the importance of the axial imidazole donor is shown when the ligand, *N*-(2-imidazol-3-ylethyl)-6-[(2-imidazol-3-ylethylamino)-methyl]-pyridine-2-carboxamide (haph), containing both an in-plane and axial imidazole donor, bonds Fe(II); such an assembly stabilizes the $(FeO)^{3+}$ chromophore and is important for the DNA cleavage action of $[Fe(haph)]^{+}$, a species which mimics the action of bleomycin [12]. Further, it has been reported that an axial imidazole donor enhances the heterolytic cleavage of H_2O_2 by a factor of 10^2 to 10^3 for Mn^{III} porphyrins [13]. Thus, it is suggested that the reactivity of Ni(II)–GlyHisHisGly(D,L) towards H_2O_2 is greater than that of Ni(II)–GlyHisHisGly(L,L).



(a) (b)

Fig. 2 Assumed configuration of Ni(II)–GlyHisHisGly(D,L) (a) and Ni(II)–GlyHisHisGly(L,L) (b)

Conclusions

Ni(II) complexes with oligopeptides containing histidine in the third position can easily scavenge O_2^- and dismutate H_2O_2 to yield O_2^-. A possible activity of Ni(II) in the dismutation of H_2O_2 is a redox role in which formation of a high oxidation state of Ni(II) is part of the catalytic cycle. That is, Ni(III) is readily accessible for the nickel complexes of the oligopeptides containing histidine in the third position, and square-pyramidal Ni(III) complexes should result from oxidation by H_2O_2.

Acknowledgements

This work was partially supported by the International Core System for Basic Research (Science and Technology Agency, Japan). We thank Professor Yoshikazu Matsushima, Kyoritsu College of Pharmacy, for his helpful suggestions.

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