CHEMISTRY

11. DMPO-mediated Conversion of Singlet Oxygen to Hydroxyl Radical in the Presence of Phenolic Compounds

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Keywords: *DMPO, singlet oxygen, hydroxyl radical, phenolic compounds, UVA*

Skin is a defensive barrier and constantly exposed to certain kinds of oxidative stress such as ultraviolet and ionizing irradiation. Singlet oxygen ($^1O_2$) is produced through the interaction of the ultraviolet-A component (UVA) of sunlight with endogenous photosensitizers such as porphyrins and flavins in the skin. Since phenolic compounds are widely used for the production of pharmaceuticals as well as cosmetic and food flavoring goods, skin is inevitably exposed to phenolic compounds. Therefore, it is considered that a phenolic compound, which is a potent electron donor, may cause the conversion of $^1O_2$ to another reactive oxygen species. Then, we intend to elucidate whether oxygen radicals are produced from $^1O_2$ in the presence of phenolic compounds.

In this study, $^1O_2$ was generated by the irradiation with UVA-visible light (λ > 330 nm) of hematoporphyrin (HP), a model compound for endogenous porphyrins in the skin and the assignment of oxygen radicals was performed by the ESR spin-trapping method using a spin trap, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO). The reaction of $^1O_2$ generated by UVA-visible light (λ > 330 nm) irradiation of air-saturated solutions of HP with phenolic compounds in the presence of DMPO gave an electron spin resonance (ESR) spectrum characteristic of the DMPO-hydroxyl radical spin adduct (DMPO/·OH). In contrast, the ESR signal of 5,5-dimethyl-2-pyrrolidone-*N*-oxyl (DMPOX), an oxidative product of DMPO, was observed in the absence of phenolic compounds. The ESR signal of DMPO/·OH decreased in the presence of ·OH scavengers, ethanol and sodium formate, and disappeared completely in the presence of sodium azide, a quencher of $^1O_2$, indicating the $^1O_2$-mediated formation of free ·OH during the reaction. A linear correlation between the amounts of DMPO/·OH produced and oxidation potentials of phenolic compounds was observed. When DMPO was replaced with 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide (DEPMPO), no DEPMPO adduct of oxygen radical species was obtained. These results indicate that $^1O_2$ reacts at first with DMPO, and the resulting DMPO-$^1O_2$ adduct is immediately decomposed/reduced to form ·OH. Phenolic compounds should participate in this reaction as electron donors.

12. Dose- and Time- Dependence of Radiation-Induced Nitric Oxide Formation in Mice as Quantified with Electron Paramagnetic Resonance

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Keywords: *nitric oxide, iNOS, X-ray irradiation, electron paramagnetic resonance, iron dithiocarbamate complex*

Nitric oxide (NO) has been shown to be an important messenger molecule in various physiological activities in animals and humans. A large amount of NO is produced in some pathological states such as sepsis, arthritis, and diabetes. There have been studies that indicate that exposing living animals to ionizing radiation caused NO formation in various organs. In cellular systems, ionizing radiation induced NO synthase in the presence of exogenous interferon-gamma. In contrast, exogenous NO has been shown to be radio-protective *in vivo*. The initial response of living systems to radiation exposure is damage in cellular components. In addition to acute damage, ionizing radiation also results in the expression of early phase inflammatory genes, although the mechanism of this gene induction is not understood. The resulting production of inflammatory cytokines is believed to cause the induction of the inducible isoform of NO synthase (iNOS) and NO formation. Free radicals that are produced by ionizing radiation are believed to act as signaling molecules to initiate inflammation. The initial step involving a inflammatory transcription factor such as nuclear factor κB (NF-κB), is followed by the expression of inflammatory cytokines and enzymes.

Although quantitative determination of radiation-induced NO in living systems it important in the evaluation of radiation trauma, is has been hampered by the unstable and elusive nature of the molecule NO. For the measurement of NO level in organs of animal models, the NO trapping method combined with electron paramagnetic resonance (EPR) spectroscopy is unique. This method utilizes the *in vivo* reaction of NO with an administered iron-sulfur cmplex that results in the formation of a stable EPR-active NO-iron complex. This complex is relatively stable in living animals and can be detected by EPR using whole-body EPR spectroscopy. But the sensitivity of the whole-body EPR spectroscopy is limited. Therefore, when the concentration of NO in the tissue is low, an organ section is subjected to *ex vivo* EPR analyses. By using this method, Voevodskaya and Vanin were the first to show that NO is produced in multiple organs in mice after whole-body gamma-ray irradiation. A lipophilic iron complex, iron-diethyl dithiocarbamate (Fe-DETC) was used as a trapping compound and the EPR measurement was made at liquid

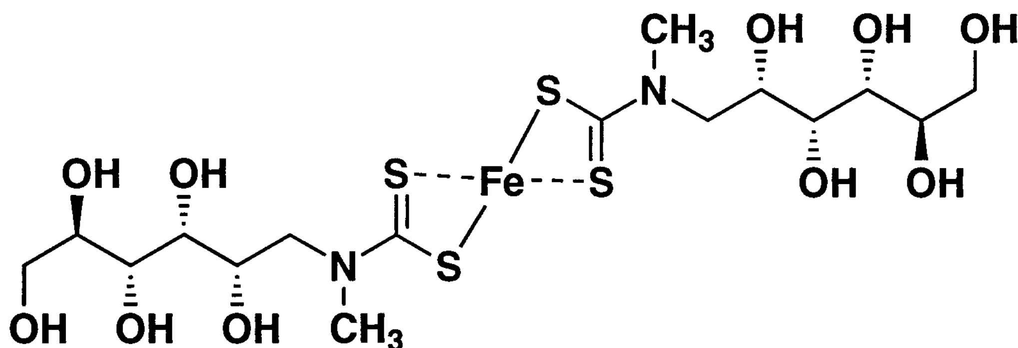

Fig. 13. Structure of Fe-MGD complex as an NO spin trap.

nitrogen temperature. Because the iron-DETC complex is not water soluble, iron and DETC had to be separately administered to the animal by different routes, thus the *in vivo* concentration of the iron-DETC complex was difficult to estimate. In this study, we used a water soluble iron complex of D-N-methyl glucamine dithiocarbamate (Fe-MGD). EPR signals from the trapped NO (NO-iron-MGD complex) were recorded in mouse liver tissue at room temperature, which allowed us to determine the accurate time course of NO formation after irradiation and the dependence of NO levels on the radiation dose. *In vivo* NO formation was quantified in mice after exposure to high-dose whole-body X-ray irradiation, ranging from 0 to 100 Gy. NO produced and accumulated in the livers of irradiated mice was determined. When mice were irradiated with 50 Gy X-rays, NO formation peaked in approximately 3 to 5 h after the irradiation was terminated. A dose-dependence study indicated that NO formation measured 5 h after irradiation leveled off for doses higher than 50 Gy. Administration of NO synthase inhibitor, N(G)-monomethyl L-arginine (L-NMMA) shortly after irradiation completely eliminated the NO signal, indicating that radiation-induced NO is produced through L-arginine-dependent NO synthase pathways. These results suggest that X-ray irradiation initiates inflammation processes, resulting in delayed NO synthase expression and NO formation.

Publication:
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13. Functional Modification of Cytochrome c by Peroxynitrite in an Electron Transfer Reaction

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Keywords: *cytochrome c, peroxynitrite, tyrosine nitration, functional modification, mitochondria, membrane potential*

Reactive oxygen species (ROS) such as superoxide and reactive nitrogen species such as nitric oxide are suggested to be involved in the pathogenesis of various diseases. Although nitric oxide and superoxide are both endogenous and important compounds for physiological responses, they become toxic and pathogenic when overproduced. Peroxynitrite, which is derived from nitric oxide and superoxide by a diffusion rate-limiting reaction, is one of the toxic intermediates produced under the ROS and RNS overproduction conditions. A typical reaction of peroxynitrite for the biological components is the nitration of protein tyrosine residues. In various diseases associated with oxidative stress, nitrotyrosine formation has been reported, suggesting the involvement of peroxynitrite in the pathogenesis. The effect of the nitration of tyrosine residue in proteins, however, is not clear. In this report, we investigated the effect of tyrosine nitration in cytochrome c on its electron transfer reaction in the mitochondrial energy production. Cytochrome c was treated with a bolus of synthetic peroxynitrite at a sub-millimolar concentration under physiological conditions, and then subjected to reduction by superoxide and oxidation by hydrogen peroxide, that is the redox reaction of cytochrome c. The ability for the membrane potential formation in the mitochondrial respiratory chain was also evaluated. After the treatment with peroxynitrite, it was revealed that the cytochrome c molecule was mono-nitrated mainly at a tyrosine residue, by liquid chromatography-electrospray ionizing mass spectrometry (LC-ESIMS) and HPLC analysis. No obvious changes were observed in the circular dichromism (CD) spectra and the absorption spectra from peroxynitrite-treated cytochrome c. These results suggest that the sub-millimolar peroxynitrite treatment did not obviously affect the cytochrome c molecule except for mono-nitration of the protein. Although the redox capacity of cytochrome c

was not changed by the treatment under the conditions in this study, it was found that the oxidation of ferrocytochrome c to ferricytochrome c by hydrogen peroxide was accelerated depending on the concentration of peroxynitrite used. When cytochrome c was treated with peroxynitrite in the presence of 5-methoxytryptamine, a selective inhibitor for the tyrosine nitration by peroxynitrite, the acceleration of hydrogen peroxide-mediated oxidation was suppressed. This result suggests that the tyrosine nitration induced the acceleration of the oxidation mediated by hydrogen peroxide, which is a non-physiological oxidation pathway of cytochrome c. Furthermore, it was also found that the formation of membrane potential in the rat liver mitochondria was suppressed when peroxynitrite-treated cytochrome c was used instead of the intact cytochrome c *in vitro*. From these results, we concluded that the sub-millimolar peroxynitrite treatment induced nitration of cytochrome c at a tyrosine residue, and that the resulting mono-nitrated cytochrome c became more susceptible to oxidation by hydrogen peroxide, concomitantly losing the ability to transfer electrons in the mitochondrial respiratory chain. We would suggest that the peroxynitrite-induced modification of cytochrome c increases the susceptibility to non-physiological oxidants, and may cause dysfunction of mitochondria by suppressing membrane potential.

Publication:
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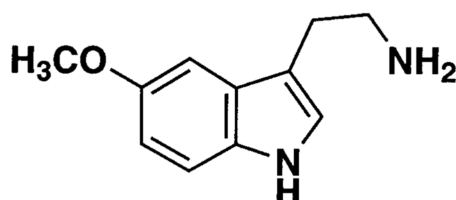
(a)



(b)

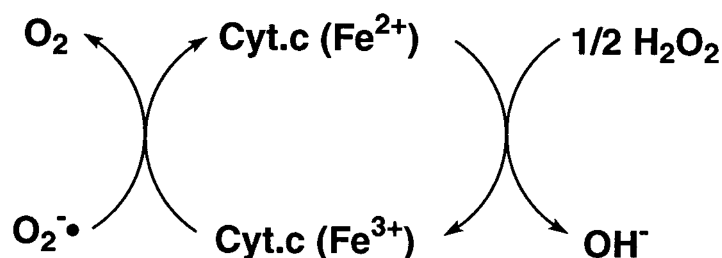


Fig. 14. (a) The structure of 5-methoxytryptamine (5MT), a nitration-selective inihibitor for peroxynitrite reaction.
(b) The redox reaction scheme for cytochrome c coupling with superoxide and hydrogen peroxide.