Detection of superoxide anion and singlet oxygen using chemical probes in biological systems during gamma irradiation

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1. Introduction

Water radiolysis means the formation of intermediates and products such as water radical cation ($H_2O•^+$), excited water (H_2O^*), hydronium ion (H_3O^+), hydroxyl radical (•OH), hydrogen ion (H^+), hydrated electron (e^-_{aq}), hydrogen radical (H•), hydrogen peroxide (H_2O_2), hydroxyl ion (OH^-), superoxide anion ($O_2•^-$), and molecular hydrogen (H_2) [1] from the dissociation of water molecules by ionizing radiation. Among them, •OH, $O_2•^-$, and H_2O_2 are biologically quite toxic and damaging to cellular components [2,3]. In addition, photosynthesis of plants produces another damaging agent, singlet oxygen ($^1O_2$), the production of which is enhanced by gamma irradiation, due to the inhibition of zeaxanthin-dependent thermal energy dissipation. Accordingly, $^1O_2$ also belongs to the reactive oxygen species (ROS) responsible for the radiation-induced biological damages. However it is difficult to detect most ROS due to the short lifetime and reactivity [4]. Therefore, the biological system under investigation a trapping compound is necessary to assay ROS, which is specifically reacts with a ROS, forming a more stable compound.

The objective of this research is to test the stability and application of trapping chemical probes to detect $O_2•^-$ or $^1O_2$ in aqueous solution during gamma irradiation using NMR, EPR, and spectrophotometric analyses.

2. Methods and Results

2.1 Chemical Probes for $O_2•^-$ and $^1O_2$

To detect $O_2•^-$ or $^1O_2$ in biological systems during gamma irradiation, radiation sensitivity of specific chemical probes, 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron) or 2,2,6,6-tetramethyl-piperidine (TEMP), and was evaluated. Also nitroblue tetrazolium (NBT) for both hydrated electron (e^-_{aq}) and $O_2•^-$ as a chemical probe was directly added to aqueous solution [5,6]. Tiron, TEMP, or NBT is a sensitive and selective chemical probe for $O_2•^-$, $^1O_2$, or e^-_{aq} and $O_2•^-$, respectively. These probes are not terribly toxic, so they are useful to *in vitro/in vivo* trapping of $O_2•^-$ or $^1O_2$. The adducts of Tiron or TEMP in reaction with $O_2•^-$ or $^1O_2$ are detectable by spin trapping.

2.2 Stability of Chemical Probes for $O_2•^-$ or $^1O_2$ to Gamma Irradiation

As mentioned above, the chemical probes may also be good candidates to detect such ROS produced in biological systems by gamma irradiation. We investigated the structural stability of Tiron and TEMP spin adducts to gamma radiations of 100-1000 Gy, which have been used to industrial irradiation for the food and agricultural products. For ^{1}H-NMR analysis, chemical probes and their spin adducts (about 10 mg) in aqueous solution after gamma irradiation were freeze-dried and re-dissolved in 1 mL of D_2O or CD_3CD. NMR spectra were recorded on a NMR spectrometer (JNM-ECA 500, Jeol Ltd., Tokyo, Japan). The result showed that the structure of TEMPO remained unaffected by gamma irradiation up to 1000 Gy (Fig. 1). Previously, we reported the stability and application of chemical probes for detection of •OH and H_2O_2 in biological systems during gamma irradiation, and also NBT was stable to gamma radiations up to 1000 Gy [7]. In contrast, Tiron and its spin adducts were broken down at 1000 Gy, implying that it can not work as a sensitive and selective probe for $O_2•^-$ with gamma radiations of 1000 Gy or above.

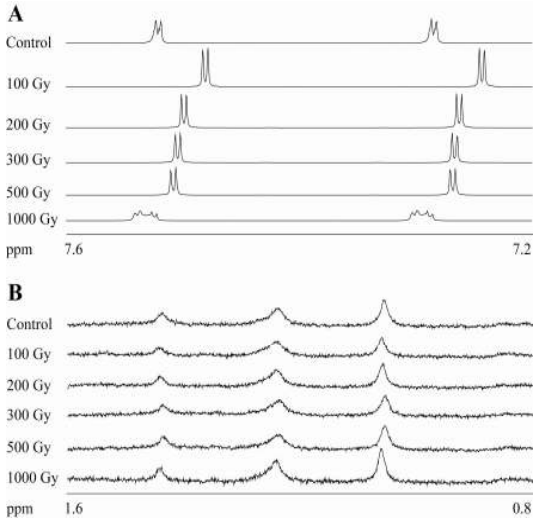

Fig. 1. NMR spectra of the probes for detection of O_2•⁻ and $^1O_2$ during gamma radiation. (A) Tiron and/or its spin adducts; (B) TEMPO.

2.3 Detection of O_2•⁻ in Aqueous Solution during Gamma Irradiation, but not $^1O_2$

Tiron forms a Tiron semiquinone radical upon reaction with O_2•⁻ [6]. The Tiron radical is an EPR-active spin adduct. When 200 mM Tiron in aqueous solution was irradiated with different doses of gamma radiation, the EPR signal from the Tiron radicals was slightly increased (Fig. 2A). These experiments demonstrate that O_2•⁻ is the major ROS produced in aqueous solution by gamma irradiation. Actually, the production of O_2•⁻ by gamma irradiation depends on the amount of O_2 dissolved in aqueous solution [1].

EPR analysis using Tiron revealed that the ROS, O_2•⁻, are not mainly produced in aqueous solution by gamma irradiation (Fig. 2A). TEMP can be reacted with another ROS, $^1O_2$, by forming TEMPO, a stable nitroxide radical yielded in the reaction of singlet oxygen [5]. This ROS is a critical damaging factor in plants exposed to excessively high light. However, the EPR signal from the TEMPO radicals was not changed in aqueous solution by gamma irradiation, while it was noticeably increased by photosensitization of riboflavin (Fig. 2B). This result suggests that $^1O_2$ cannot be substantially produced in biological systems by gamma irradiation.

This trend was dose-dependently manifested in O_2-saturated aqueous solution using nitroblue tetrazolium (NBT), a common probe for both hydrated electron (e^-_{aq}) and O_2•⁻ (date not shown).

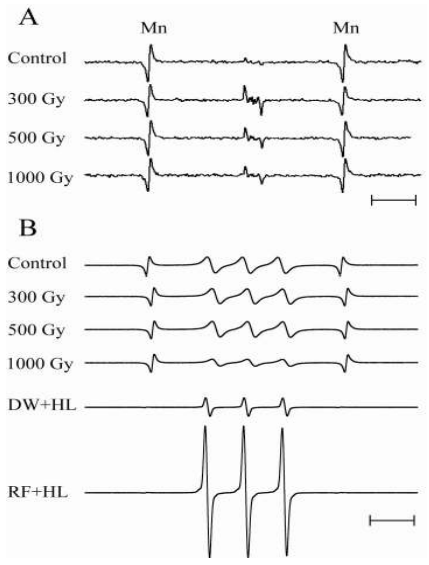

Fig. 2. EPR spectra of Tiron and TEMP spin adducts in aqueous solution during gamma irradiation. (A) Tiron semiquinone radicals; (B) TEMPO. Riboflavin (RF) was used to produce $^1O_2$ by photosensitization under the high light (HL). Bar, 2 mT.

3. Conclusions

In the present study, we demonstrated that O_2•⁻ or $^1O_2$ are not the main ROS produced by water radiolysis. In addition, considering the structural stability and specificity of chemical probes were tested. We suggest that Tiron and NBT or TEMP could be utilized as semi-quantitative chemical probes to estimate the level of O_2•⁻ or $^1O_2$ in biological systems during gamma irradiation.

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