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Chemiluminescence Study of the Formation of Protein Radicals Under the Influence of Ultraviolet-B Rays

Abstract

The presented work reviews the molecular mechanisms of the formation of reactive oxygen species (ROS) in protein solutions exposed to ultraviolet-B (UV-B) (mainly UV-B: 280–315 nm) radiation, the role of proteins in their formation, and the biological significance of these processes. It is known that ROS species, in turn, cause the formation of long-lived protein radicals (LLPRs). The amount of LLPRs formed in human serum albumin protein upon exposure to UV-B (1.2×10^2, 2.4×10^2, 3.6×10^2, and 4.8×10^2 erg/mm^2) irradiation was studied by the chemiluminescence (XL) method. It was observed that the intensity of XL varies depending on the irradiation dose. The introduction of antioxidants into the system leads to a weakening of the effect of UV radiation. Thus, inosine neutralizes long-lived protein radicals more effectively than natural antioxidants. In our opinion, since ROS play a signaling and regulatory role in biological studies, their formation and LLPRs play an important role in the adaptation of living systems to stress factors.

Keywords: *Long-lived protein radicals, oxidative stress, reactive oxygen species, UV-B radiation, chemiluminescence*

Introduction

Free radicals, defined as atoms or molecules containing one or more unpaired electrons, are highly chemically reactive species capable of inducing destructive effects on molecular components within biological systems (Ayala et al., 2014; Kowalczyk, Sulejczak, et al., 2021; Mandal et al., 2022). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) can originate from endogenous metabolic processes or be induced by exogenous stressors such as ionizing radiation, ultraviolet radiation, environmental pollutants, cigarette smoke, heavy metals, and certain pharmacological agents (Kocharli & Hummatova, 2020). During normal cellular respiration, approximately 1 –2% of molecular oxygen is converted into ROS. When the concentration of reactive oxygen species exceeds the buffering capacity of antioxidant defense systems, cascade reactions can occur, resulting in lipid peroxidation, protein denaturation, and DNA damage (Lobo et al., 2010; Ayala et al., 2014; Zorov et al., 2014; Kowalczyk, Sulejczak, et al., 2021).

Although UV-B radiation constitutes a minor fraction of solar radiation, it exhibits strong photobiological activity. It can induce the formation of pyrimidine dimers in DNA and structural alterations in proteins. Proteins, due to their abundance in biological systems, are particularly vulnerable to oxidative stress and become primary targets for radical-mediated damage. Although UV-B radiation constitutes a minor fraction of solar radiation, it exhibits strong photobiological activity. It can induce the formation of pyrimidine dimers in DNA and structural alterations in proteins. Proteins, due to their abundance in biological systems, are particularly vulnerable to

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oxidative stress and become primary targets for radical-mediated damage (Rastogi, Kumar, et al., 2010; Kocharli & Hummatova, 2019).

Free radicals and other oxidizing agents are involved in various physiological processes and also contribute to the development of diseases. Such modifications are not only implicated in the loss of protein function but also play a critical role in the pathogenesis of aging and degenerative diseases. Protein oxidation, driven by reactive radicals, disrupts cellular signaling and impairs homeostasis (Alugoju et al., 2014; Bin et al., 2017; Jomova, Raptova & Alomar, 2023; Chandimali, Bak, et al., 2025).

Upon exposure to UV-B radiation, ROS species such as singlet oxygen ($^1O_2$) and superoxide anion ($\bullet O_2^-$) are formed, leading to site-specific post-translational modifications, particularly at cysteine and methionine residues. Cysteine residues, as one of the primary targets of ROS, undergo selective oxidation that alters protein structure and function and promotes the formation of various secondary products (Brosnan et al., 2006; Alugoju et al., 2014; Rajesh et al., 2010).

ROS produced in bovine serum albumin and gamma-globulin solutions via laser irradiation lead to the formation of LLPRs with a half-life of approximately ~4 hours. LLPRs generated by laser irradiation last for a long time or several hours and result in the formation of ROS — hydrogen peroxide, hydroxyl, and superoxide radicals (Vladimir et al., 2017).

Studies have shown that LLPRs can be generated not only by ionizing radiation but also by UV irradiation, peroxynitrite ($ONOO^-$), uranyl ions (UO_2^{2+}), decomposition of H_2O_2 by peroxidases, and hyperthermia (Michael, 2005; Gudkov et al., 2010). Using highly specific techniques such as electron spin resonance (ESR) spectroscopy and chemiluminescence, the lifetime of these radicals has been revealed to exceed 20 hours (Yoshimura et al., 1993). Furthermore, these protein radicals may interact with other biomolecules, particularly DNA, thereby contributing to genotoxic damage (Bruskov, Popova, et al., 2014).

Materials and Methods

Albumin protein solution was selected as the research object, and the formation of LLPRs was measured using a quantum chemiluminescence detector. Ascorbic acid and inosine were used as natural antioxidants in the experiments. The chemiluminescence method, a highly sensitive technique for detecting energy emission during radical reactions, was employed (Kocharli & Hummatova, 2024). UV-B irradiation was performed using a PRK-4 mercury lamp, and protein samples were exposed to radiation doses of 1.2×10^2, 2.4×10^2, 3.6×10^2, and 4.8×10^2 erg/mm².

Results and Discussion

It has been established that long-lived protein radicals can be a source of reactive oxygen species and long-term oxidative stress in biological systems and can transmit oxidative damage to other molecules, including DNA. Long-lived protein radicals can be measured immediately using electron spin resonance (ESR) spectroscopy. However, its low sensitivity often necessitates the use of high radiation doses (1–5 kGy) when employing the chemiluminescence method (Luxford, 1999; Gudkov et al., 2007). The CL method, which is a sensitive method for detecting free radical reactions, releases energy in the form of light quanta during the interaction of radicals (Kocharli & Hummatova, 2020).

In this study, we assessed the LLPR formation by measuring the chemiluminescence of protein solutions exposed to UV-B radiation using a highly sensitive chemiluminescence method. It is known that some natural antioxidants, such as guanosine, inosine, and ascorbic acid, can neutralize the oxidative stress caused by the oxidation of free radicals. Model proteins, including egg albumin, bovine serum albumin, HSA, immunoglobulin G, and histone H1, are well-established systems for studying LLPR generation (Gudkov et al., 2010).

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Figure 1. Scheme of the formation of Long-Lived Protein Radicals during exposure to UV-B rays.

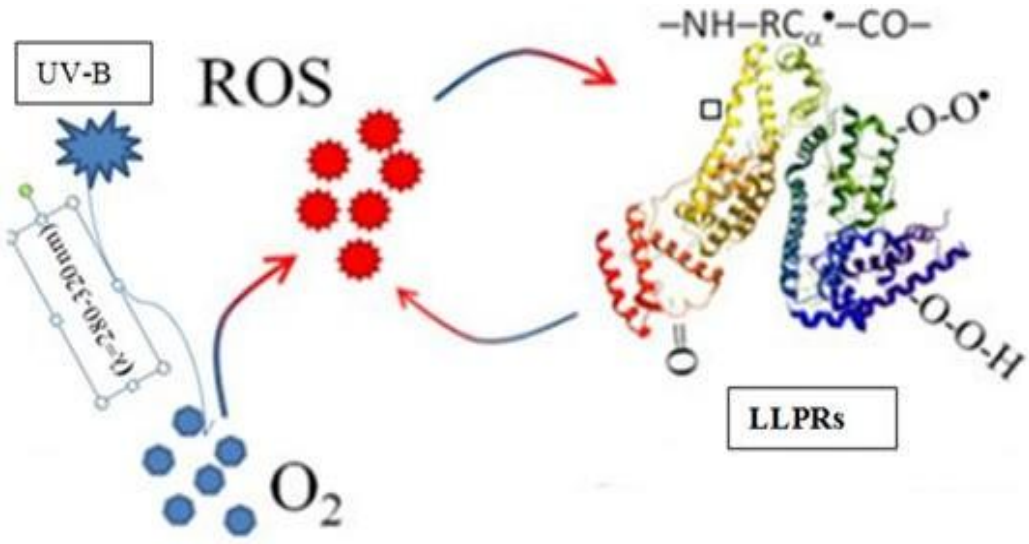
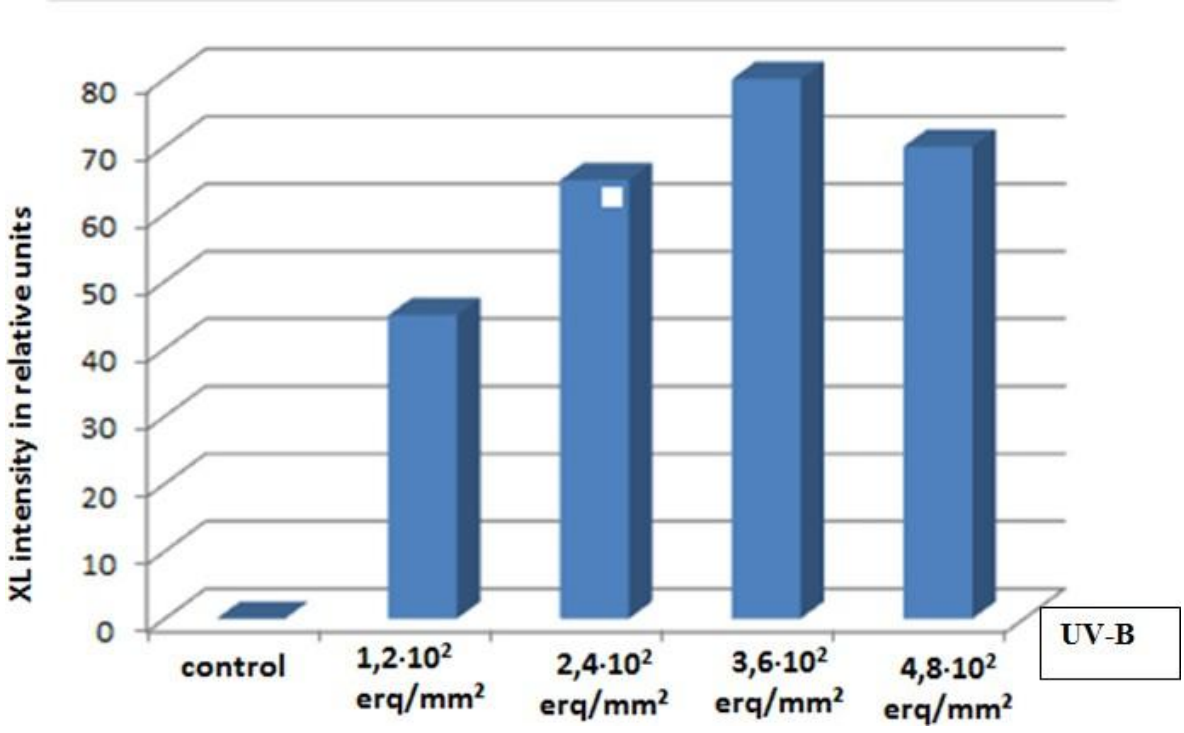


Figure 2. Changes in chemiluminescence (CL) intensity in human serum albumin solution (1%) under different doses of UV-B radiation (1.2×10² – 4.8×10² erg/mm²).



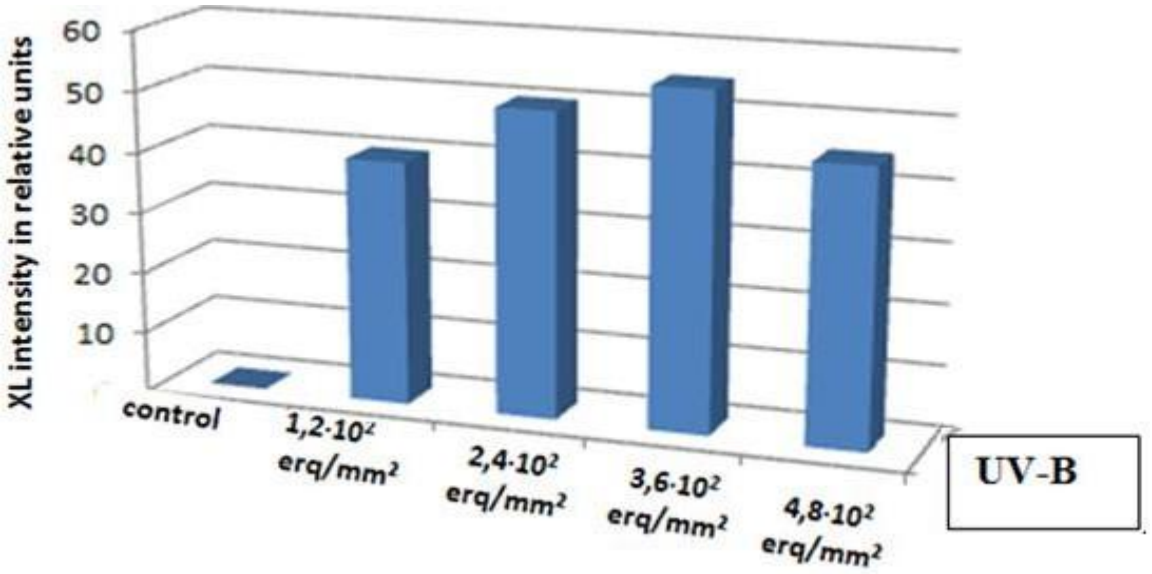
As shown in Figure 2, an increase in chemiluminescence intensity was observed in albumin solutions exposed to UV-B radiation at doses ranging from $1{,}2 \cdot 10^2$-$3{,}6 \cdot 10^2$ erg/mm². As shown in Figure 2, an increase in chemiluminescence (CL) intensity was observed in albumin solutions exposed to UV-B radiation at doses ranging from 1,2×10² to 3,6×10² erg/mm². Thus, the maximum value of the CL intensity was observed in the albumin solution exposed to a dose of 3,6×10² erg/mm² UV-B radiation. However, at a higher dose of 4,8×10² erg/mm², a decrease in CL intensity was noted.

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We propose that the changes in the CL induction curve during UV-B exposure are associated with the formation of long-lived protein radicals (LLPRs) generated in vitro during radiation exposure.

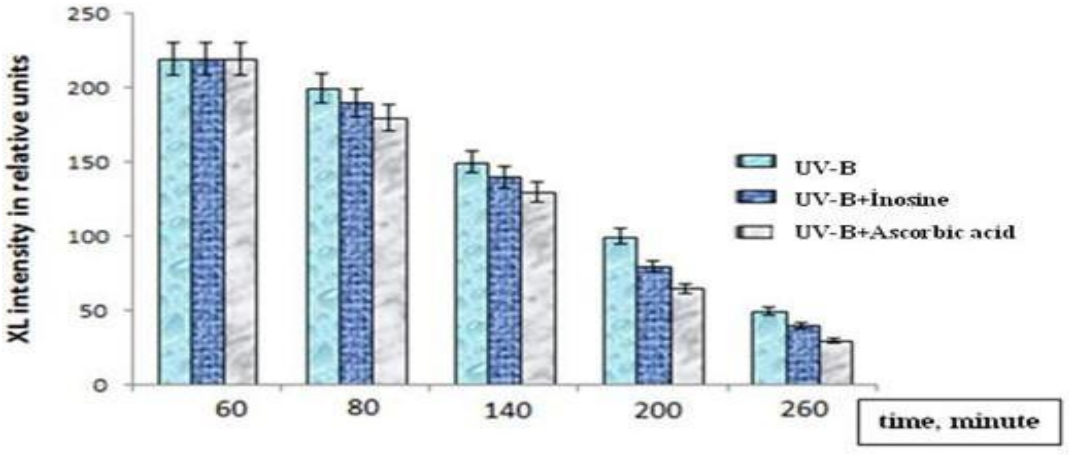
As shown in Figure 3, after exposure of the albumin solution to inosine (1×10-4 M) resulted in decreased CL intensity following irradiation with UV-B at doses ranging from 1,2×10² to 3,6×10² erg/mm² (Davies, 2005).

Figure 3. Effect of inosine (1×10^{-4} M) on the amount of LLPRs formed upon UV-B radiation of human serum albumin protein (1%).



The most significant reduction occurred at a high dose of the stress factor, which is due to the neutralizing effect of inosine on LLPRs formed in human serum albumin (Yoshimura, Matsuno & Miyazaki, 1993).

Similar results were obtained in experiments with the effect of ascorbic acid (1×10^{-3} M). Figure 4 illustrates the neutralization of LLPRs by antioxidants such as ascorbic acid and inosine. Based on these findings, we further examined the concentration-dependent effects of inosine at different concentrations on the elimination of LLPRs formed in human serum albumin upon UV-B exposure. Following the addition of inosine (riboxin) and ascorbic acid to human serum albumin, a significant reduction in long-lived protein radicals (LLPRs) was observed within 20 minutes. After 200 minutes of incubation, inosine reduced the LLPR content by approximately 2.5-fold, while ascorbic acid achieved a 1.5-fold reduction (Phaniendra, Jestadi & Periyasamy, 2015).



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Conclusion

These results indicate that inosine is more effective than ascorbic acid in neutralizing long-lived protein radicals among the natural antioxidants tested. It is assumed that the findings of this study may contribute to a better understanding of the mechanism of highly reactive molecules and their role as mediators of oxidative modifications of cellular components.

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