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Figure 1

Figure 1: Figure 1

Abstract

Full Text

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INTERACTION OF RADICALS OF IRRADIATED PROTEIN AND POLYMETHYL METHACRYLATE WITH OXYGEN AND ALKYLPHENOLS

The study of the mechanisms of the primary biological action of radiation is one of the principal directions in radiobiology. The question of the role and participation of free radicals of irradiated proteins and nucleic acids (the most important biochemical components of cells) is of especially substantial significance. The study of the chemical properties of such radicals, their reactivity, the elucidation of the possibility that these radicals initiate radical (chain) reactions, and the possibility that they cause undesirable biochemical shifts leading to disruption of cellular metabolism—all this will make it possible to approach more closely the problem of the mechanism of the biological action of radiation and the rational selection of protective and therapeutic agents. In 1957 ([1]), one of us with coworkers proposed using certain low-toxicity inhibitors of free-radical processes for the prevention and treatment of radiation sickness in experiments. Subsequent studies ([2-7]) did indeed show that such inhibitors, in experiments on various models and with irradiated animals, possess a pronounced protective action. It was therefore important to study the reactions of radicals of irradiated proteins with various substances—oxygen and inhibitors of free-radical reactions.

Fig. 1. Kinetics of the change in the relative intensity of chemiluminescence (solid curve) and kinetics of the change in the relative concentration of radicals (points on the curve) upon dissolution of irradiated PMMA in 1,2-dichloroethane

In numerous studies using the EPR method it has been established that radicals arise upon irradiation of proteins ([8-11]). It has also been shown that, under the action of oxygen, water, and certain radioprotective substances on such radicals, the intensity and form of the EPR spectrum of the radicals change ([12-16]).

The aim of the present work was to study the reactions of radicals of irradiated serum albumin. In parallel with the investigation of the properties of protein radicals, the same reactions of radicals of irradiated polymethyl methacrylate

Figure 2

Figure 2: Figure 2

Figure 3

Figure 3: Figure 3

(PMMA) were studied. In preliminary experiments we had observed measurable luminescence upon dissolving irradiated protein and PMMA. Therefore, a comparative study of radical reactions by two different methods—EPR and chemiluminescence—was undertaken.

Solvents were added to the irradiated substances, and the kinetics of radical decay was studied on an EPR apparatus, while the kinetics of luminescence decay was studied on an apparatus for recording weak luminescence. Protein and PMMA were irradiated in previously evacuated ampoules on a GUT-Co-400 installation with doses of···

5-20 Mrad. When experiments were carried out on the EPR-2 apparatus of the Institute of Chemical Physics, the irradiated substances were transferred into ampoules and the EPR spectra of these substances were recorded. Then solvents were added to the same ampoules: glacial acetic acid in the case of protein and 1,2-dichloroethane in the case of PMMA, and the decrease in the number of radicals with time was recorded. In studying the kinetics of chemiluminescence, the irradiated substances were placed in a reaction vessel located in front of a photomultiplier. The photocurrent was amplified, and the kinetic curves of the luminescence arising after addition of solvents to the irradiated substances (water and glacial acetic acid to the protein, dichloroethane to PMMA) were recorded on an EPP-09 electronic potentiometer. Provision was made for carrying out experiments in vacuum (10^{-3} mm Hg) and in air.

Fig. 2. Increase in chemiluminescence intensity upon admission of oxygen during the course of the process: *a*—irradiated protein in glacial acetic acid, *b*—irradiated protein in water, *v*—irradiated PMMA in dichloroethane

The EPR spectrum of irradiated protein is an unresolved doublet with a splitting of about 12 oersteds ([9]). The EPR spectrum of irradiated PMMA also corresponded to the data of previous works ([17,18]).

Fig. 3. Kinetic luminescence curves upon dissolution in air of irradiated serum albumin: *a*—in glacial acetic acid, *b*—in an ionol solution

Figure 1 presents the dependence of the relative intensity of chemiluminescence on time after addition of dichloroethane to irradiated PMMA (solid curve) and the dependence of the relative concentration of radicals on time, obtained by the EPR method (points); it is seen that the curves coincide satisfactorily. The kinetics of the decrease in the relative concentration of protein radicals corresponds to the kinetics of first-order processes ([19]). In different experiments the

Fig. 4. Kinetic luminescence curves upon dissolution in air of irradiated PMMA. *a*—in dichloroethane, *b*—in an ionol solution

Figure 4: Fig. 4. Kinetic luminescence curves upon dissolution in air of irradiated PMMA. *a*—in dichloroethane, *b*—in an ionol solution

values of the constants proved to be 0.95–$2.3 \cdot 10^{-2}$ s^{-1}. The kinetic curve of luminescence on the descending part of the curve upon addition of acetic acid and water to irradiated protein in vacuum also corresponds to first-order kinetics, with experimental values of the constants 1.3–$2.5 \cdot 10^{-2}$ s^{-1}. The coincidence of the data obtained by the EPR method and by the chemiluminescence method indicates that the radicals of the irradiated protein released upon dissolution are responsible for the luminescence (luminescence arises upon their recombination).

In work (20) it was shown that, when radicals R· are replaced by radicals RO_2· in hydrocarbon oxidation processes, the intensity of chemiluminescence increases greatly. It could be expected that in our case as well, upon addition of oxygen, the intensity of luminescence would increase.

Fig. 4. Kinetic luminescence curves upon dissolution in air of irradiated PMMA. *a*—in dichloroethane, *b*—in an ionol solution.

Figure 2 shows the dependence of the chemiluminescence intensity on time when glacial acetic acid was added to irradiated protein in vacuum (upper plot). During the course of the process the system was brought into contact with air (indicated by an arrow). As can be seen, the luminescence intensity increased several-fold. The middle plot presents the dependence of the chemiluminescence intensity on time after water was added to irradiated protein in vacuum.

In this case luminescence also arises, which flares up when oxygen is admitted. The lower plot shows the analogous dependence for irradiated PMMA. In all cases the luminescence intensity increases, which supports the assumption that radicals R· are replaced by radicals RO_2·. If this is true, then in the presence in the system of substances that interact selectively with peroxide radicals (for example, in the presence of 2,6-di-tert-butyl-4-methylphenol (ionol)), the chemiluminescence intensity in experiments carried out in air should decrease.

Figures 3 and 4 give the corresponding kinetic curves. It is clearly seen that both in the case of the irradiated protein—acetic acid system and in the case of irradiated PMMA—dichloroethane, the luminescence intensity decreases sharply in the presence in the system of an inhibitor effectively interacting with radicals RO_2·.

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Note: Figure translations are in progress. See original paper for figures.