PHYSICAL CHEMISTRY

1964

SovietRxiv

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Abstract

Full Text

PHYSICAL CHEMISTRY

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LONG-LIVED AFTERGLOW OF AQUEOUS SOLUTIONS OF PROTEINS AND SYNTHETIC POLYMERS IRRADIATED WITH X-RAYS

The authors of a number of studies ([1–2]) discovered the phenomenon of long-lived afterglow of dry proteins and protein solutions under the action of ultraviolet light. Assumptions were advanced concerning the mechanism of this effect; it was shown that the afterglow is apparently associated with recombination reactions of peroxide radicals formed under the action of radiation ([5]). Long-lived chemiluminescence was also found upon dissolution of γ-irradiated proteins, caused by recombination of peroxide radicals ([3]).

It was natural to suppose that, under the action of X-radiation on protein solutions, with sufficiently high sensitivity of the photometric apparatus, effects of recombination afterglow could be observed. It should be noted that at low dose rates of irradiation the stationary concentration of radicals is small and therefore, even at high values of the recombination rate constants, the lifetime of the radicals must be sufficiently long.

Fig. 1. Kinetic curves of the afterglow of solutions of proteins and polymers irradiated with X-rays: 1 —polyethylene oxide, 2 —polyvinylpyrrolidone, 3 — bovine serum albumin; 4 —hemoglobin and their anamorphoses.

Proceeding from these premises, we undertook an attempt to detect afterglow in aqueous solutions of proteins and synthetic polymers. It turned out that, when solutions are irradiated at room temperature, at doses of 1–10 krad, a weak glow

Fig. 2 and Fig. 3 graphs

Figure 2: Fig. 2 and Fig. 3 graphs

arises, continuing after the action of the radiation for hundreds of seconds.

The experiments were carried out in a flow-through apparatus. The solution was irradiated in a thermostated vessel ($t = 25^{\circ}$) with X-rays (on an RUT-200 apparatus), and was then rapidly pumped into a thermostated cuvette located near the photocathode of an FEU-33 photomultiplier. The photocurrent was amplified, and the kinetic curves of the afterglow were recorded on an EPP-09 electronic potentiometer.

The dose rate of irradiation was 77 rad/sec. The photomultiplier was surrounded by a layer of lead. As a relative standard signal I_{cc}, the background of the multiplier cathode produced by irradiation was used. The experiments were carried out with both aqueous and buffer solutions of proteins and polymers.

Six proteins were selected as objects of study (bovine serum albumin, hemoglobin, hyaluronidase, ribonuclease, trypsin,

cytochrome C) and 3 water-soluble polymers (polyethylene oxide, polyvinylpyrrolidone, polyvinyl alcohol).

Figure 1 presents the dependences of the photocurrent I/I_{cc} on time after irradiation of a 1% polyethylene oxide solution (1), a 5% polyvinylpyrrolidone solution (2), a 0.5% serum albumin solution (3), and a 0.5% hemoglobin solution (4), and, respectively, their anamorphoses $\left(\sqrt{I_{cc}/I}\right.$ as a function of time), irradiated for 1 minute.

Fig. 2. Dependence of the relative afterglow intensity of a serum albumin solution on temperature

Fig. 3. Effect of the irradiation dose on the luminescence intensity of a serum albumin solution

As can be seen from Fig. 1, the kinetic curves are, for the most part, described by kinetic equations for second-order processes, if it is assumed that the luminescence intensity is proportional to the square of the radical concentration. Similar dependences were obtained for solutions of trypsin, hyaluronidase, and ribonuclease. The luminescence intensity of solutions of cytochrome C and polyvinyl alcohol is very small.

We carried out a more detailed study of the kinetic characteristics of afterglow for solutions of bovine serum albumin. Dependences were obtained of the relative intensity 1 min after irradiation on temperature, dose, and concentration.

Figure 2 presents the temperature dependence of the relative afterglow intensity in Arrhenius coordinates. The value of the activation energy was 8.5 ± 2 kcal/mol.

Fig. 4 graph

Figure 3: Fig. 4 graph

The luminescence intensity increases with the irradiation dose (Fig. 3). It is interesting that already at doses of 500–1000 rad, afterglow effects can be observed.

Fig. 4. Change in intensity as a function of serum albumin concentration

The dependence of the intensity on the protein concentration (Fig. 4) is nonlinear. The character of the curve suggests that the afterglow effect is associated with the indirect (through OH and HO_2) action of the radiation.

We also carried out experiments on the effect of oxygen, cysteine, and inhibitors of free-radical reactions (propyl gallate and isopropyl gallate) [4] on the afterglow process. It turned out that bubbling the protein solution with argon for 10–15 min before irradiation leads to a decrease in the luminescence intensity by a factor of 1.5–2. Addition of cysteine at a concentration of 10^{-4} mol/l before irradiation completely removes the afterglow effect. Cysteine similarly affects the afterglow of solutions of synthetic polymers. Introduction into the system of inhibitors—propyl gallate and iso-

propyl gallate leads to a decrease in the luminescence intensity. The addition of cysteine after irradiation also decreases the luminescence intensity.

Thus, the experimental data presented make it possible to conclude that the afterglow effect upon irradiation of solutions with X-rays is analogous to the afterglow effect upon irradiation with ultraviolet light. Apparently, the afterglow is caused by the recombination of peroxide radicals of proteins and polymers.

Further study of the mechanism of afterglow processes is of great interest for radiation biology: first, because it is possible to observe reactions involving protein molecules at low irradiation doses, when denaturation effects are still small; second, because the study of the influence of various substances on afterglow may make it possible to create a test for the preliminary selection of radioprotective substances.

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Received
16 VIII 1964

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