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Abstract

Full Text

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ON THE NATURE OF THE ULTRAWEAK LUMINESCENCE OF IRRADIATED SOLUTIONS OF DEOXYRIBONUCLEIC ACID (DNA)

In our previous work, the rate of decomposition of hydroperoxide compounds in irradiated DNA solutions was studied, and it was shown that two types of hydroperoxides are formed, differing in stability ([1]). The use of the chemiluminescence method made it possible to record ultraweak luminescence in irradiated DNA solutions, the intensity of which varied with time according to an exponential law ([2]).

It is known that chemiluminescence arises in the course of many chemical processes. It has been shown that the elementary act of luminescence accompanying oxidative reactions is the recombination of peroxide radicals, and that the luminescence intensity is proportional to the rate of the elementary reaction leading to their formation ([3]).

In elucidating the mechanism of various biochemical processes, the ultraweak luminescence of biological systems is studied (living roots, tissue homogenates, yeast cells). It has been found that chemiluminescence arises in reactions of autoxidation of various biosubstrates—tissue lipids, amino acids, carbohydrates ([4]), during biological oxidation of fatty acids in mitochondria and microsomes of animal tissues, and in photosynthetic reactions ([5]).

By the chemiluminescent method in combination with the EPR method, the elementary reaction of interaction of radicals of irradiated protein with molecules of one of the inhibitors of free-radical processes—2,6-di-tert-butyl-4-methylphenol (ionol)—was studied ([6]).

The aim of the present work was to study the nature of the ultraweak luminescence arising in irradiated DNA solutions.

In the work we used 0.05–0.1% aqueous solutions of native DNA preparations isolated from calf thymus (ε_p 6500–6600), and high-polymer dry DNA preparations manufactured by the Worthington Biochemical Corporation. Aerated solutions were irradiated with doses of 28–85 kr on a RUT-200-20-3 apparatus at 22°. The luminescence was recorded on a photometric setup. The luminescence detector was a FEU-39 photomultiplier, cooled with solid carbon dioxide, with a sensitivity interval of 200–600 mμ. The luminescence of irradiated DNA

Figure 1 and Figure 2

Figure 1: Figure 1 and Figure 2

solutions is detected at 25° and increases when the temperature is raised. The luminescence intensity decreases with time according to an exponential law over the course of 150-300 sec. In Fig. 1A, the kinetic luminescence curve of an irradiated DNA solution at 75° and its semilogarithmic anamorphosis are presented, from whose slope the value of the rate constant for luminescence decay, k_{cl}, was found. The values of k_{cl} at 75° for different DNA preparations lie in the interval $(0.8–2.0) \cdot 10^{-2}$ sec^{-1}.

It could be assumed that in this case, as in a number of chemical reactions, the luminescence is associated with the presence of hydroperoxide compounds in the system. The validity of this assumption was demonstrated in studying the relationship between the luminescence intensity and the kinetics of decomposition of hydroperoxide compounds of DNA, as well as in studying the luminescence of DNA solutions irradiated in an argon atmosphere.

In Fig. 1A the change with time in the intensity of luminescence and the concentration (optical density) of the less stable hydroperoxide in the irradiated DNA solution is compared (points on the decay curve of the luminescence intensity). As can be seen, the points lie well near the curve, i.e., the intensity of chemiluminescence and the concentration of hydroperoxide change with time according to one and the same law, with one and the same value of the constant.

Upon irradiation of DNA solutions in an atmosphere of argon (the solutions were purged with argon for 45 min before irradiation), hydroperoxides were not detected by analytical methods, and only insignificant luminescence was observed.

Further it was shown that the maximum intensity

Fig. 1. A—change with time in the intensity of luminescence (1) and the concentration (optical density D) of the less stable hydroperoxide in an irradiated DNA solution (points on curve 1) at 75° (pH 6.4); 2—semilogarithmic anamorphosis of curve 1. B—dependence of the maximum luminescence intensity on the concentration of hydroperoxides in an irradiated DNA solution

Fig. 2. Dependence of the rate constant of luminescence decay on temperature in the coordinates $\lg k \ - (10^3/T)$. 1—native DNA preparation; 2—dry DNA preparation

of luminescence $I_{\max}$ depends linearly on the concentration of hydroperoxides in the irradiated DNA solution (Fig. 1B). Different concentrations of hydroperoxides in 0.07% DNA solutions were obtained by applying different irradiation doses (the concentration of DNA hydroperoxides increases linearly in the dose interval 28-70 kr) or by diluting the irradiated DNA solution several times. The rate constant of luminescence decay does not depend on the initial con-

centration of hydroperoxides, i.e., first-order reaction kinetics with respect to the initial concentration of hydroperoxides is observed. Thus, the intensity of luminescence at any given moment of time is determined by the concentration of DNA hydroperoxides present in the system under study.

The dependence of the rate constant of luminescence decay on temperature was studied in the range 40-90°. The dependence obtained is well linearized in the coordinates $\lg k \ -1/T$ (Fig. 2). The value of the activation energy calculated from these data is 13 ± 1 kcal/mole and practically coincides with the value of the activation energy for the decomposition of the less stable hydroperoxide in an irradiated DNA solution, measured by the chemical method (1). This comparatively low value of the activation energy for the thermal decomposition of hydroperoxides may apparently be associated

because what occurs here is a catalytic decomposition of hydroperoxides, which, however, requires further study.

It is known that the properties of native DNA change little when the pH of the medium is varied in the range 4-11. Since under the conditions of our experiments the DNA samples were subjected to considerable effects of irradiation and heating, one could expect a noticeable influence of pH on the character of the luminescence. After irradiation of DNA solutions, the pH of the medium was varied with acetate or phosphate buffers (0.05 M) from 2 to 10. The dependence of the ratio of the chemiluminescence decay-rate constant at a given pH to the value of the constant at pH 7 ($k_{\mathrm{pH}\,7}$) on the pH value is shown in Fig. 3. As is seen from the figure, in the interval pH 6.2-6.8 a sharp maximum of the constant values is observed, while outside this interval the constant changes only slightly. This peculiar phenomenon still requires explanation. It is possible that pH affects the rate of decomposition of hydroperoxides. In addition to the change in the value of the constant k_{cl}, a regular change with pH in the value of $I_{\max}$ is also observed. As a result, the total light yield (the area under the chemiluminescence curve) changes with pH. Luminescence is practically absent at pH below 2; increasing the pH from 2 to 7 leads to a gradual increase in the total light yield, and upon transition to an alkaline medium, a slight change in pH from 7.0 to 7.5 gives a sharp increase in the light yield. Such a strong influence of pH on the character of chemiluminescence requires strict maintenance of specified pH values when experiments are carried out.

Fig. 3

Fig. 4

Fig. 3. Dependence on the pH of the medium of the ratio of the luminescence decay-rate constant at a given pH to the rate constant at pH 7 (68°)

Fig. 4. Curve of the dependence of the maximum chemiluminescence intensity on the concentration of propyl gallate (A) and its anamorphosis in the coordinates $\sqrt{I_0/I_{\max}}$–inhibitor concentration (B) (68°, pH 7.1)

As in the case of chemiluminescence in ordinary chemical reactions, it could be

assumed that in irradiated DNA solutions the luminescence also arises upon recombination of radicals (most probably peroxide radicals). In this case the luminescence intensity should be proportional to the square of the radical concentration: $I \sim [\mathrm{R}]^2$. The introduction of inhibitors of free-radical reactions should reduce the intensity of chemiluminescence as a consequence of a decrease in the radical concentration:

$$\sqrt{I_0/I} = [\mathrm{R}]_0/[\mathrm{R}],$$

where I_0 and $[\mathrm{R}]_0$ are the luminescence intensity and radical concentration in the absence of inhibitor, and I and [R] are those in the presence of inhibitor. Since the ratio $[\mathrm{R}]_0/[\mathrm{R}]$ depends linearly on the inhibitor concentration, the quantity $\sqrt{I_0/I}$ should also increase linearly with increasing inhibitor concentration (7). Indeed, a considerable decrease was found

intensity of the chemiluminescence of the irradiated DNA solution in the presence of inhibitors of free-radical reactions. Normal propyl ester of gallic acid (propyl gallate) was used as the inhibitor. A solution of propyl gallate ($2.5 \cdot 10^{-4}$—$1.0 \cdot 10^{-3}$ M) in phosphate buffer was introduced into the irradiated DNA solution (final buffer concentration 0.05 M). Fig. 4 presents the curve of the dependence of the chemiluminescence intensity on the concentration of propyl gallate (A), and the anamorphosis of this curve in the coordinates $\sqrt{I_0/I_{\max}}$ – inhibitor concentration (B). I_0 and $I_{\max}$ were measured at identical values of the concentration of DNA hydroperoxides in the solution, i.e., at the same rate of initiation of free radicals. In this case, the linear character of this anamorphosis makes it possible to suppose that the chemiluminescence of irradiated DNA solutions has a radical character.

Special experiments showed that hydrogen peroxide ($1 \cdot 10^{-4}$ M) and protein (4—$6 \cdot 10^{-4}$ wt.%), present in the irradiated DNA solution, do not exert a substantial influence on the luminescence, and that the oxygen concentration in an already irradiated DNA solution has little effect on the luminescence intensity in a neutral medium.

Thus, it may be considered that the luminescence of irradiated DNA solutions is chemiluminescence having a free-radical nature and caused by the presence in the system of DNA hydroperoxide compounds.

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