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Figure 1 and Figure 2

Figure 1: Figure 1 and Figure 2

Abstract

Full Text

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KINETIC CHARACTERISTICS OF THE DECOMPOSITION OF PEROXIDE COMPOUNDS IN IRRADIATED DNA SOLUTIONS

The formation of peroxides in biological systems under the action of ionizing radiation has been observed by many investigators. It has been noted that peroxide compounds may play an essential role in processes of depolymerization and oxidative decomposition of large molecules, and in the development of chain oxidation reactions. Of particular interest is the formation of peroxides in high-polymer molecules of nucleic acids, especially from the standpoint of their possible role in the phenomenon of radiation aftereffect.

Fig. 1. *a* –kinetic curves of the change in the concentration of hydroperoxide compounds in irradiated DNA solutions. 1 –52°, 2 –62°, 3 –72°, 4 –79°. *b*, *c* – semilogarithmic anamorphosis of the kinetic curve of the change in concentration at 52° of hydroperoxides (*b*) and of the less stable among them (*c*)

Fig. 2. Arrhenius dependence of the rate constants for decomposition of hydroperoxide compounds in irradiated solutions: *a* $-k_1$ of DNA, *b* $-k_2$ of DNA, *c* $-k_{\text{hp}}$ of thymine

In a number of works it was shown that, upon irradiation of aqueous DNA solutions, hydroperoxides of pyrimidine bases are formed ([1,2]). However, these investigations were purely qualitative in character.

Recently we succeeded in recording an extremely weak luminescence in irradiated DNA solutions which, apparently, is due to the decomposition of peroxide compounds of DNA ([3]). Studying the change in luminescence at different temperatures, we obtained preliminary quantitative characteristics of the process

Fig. 3. Kinetic curves of hydroperoxide decomposition in irradiated solutions of thymine (1-4) and cytosine (5) and their semilogarithmic transformations. 1 and 5–52°, 2–62°, 3–72°, 4–79°

Figure 2: Fig. 3. Kinetic curves of hydroperoxide decomposition in irradiated solutions of thymine (1-4) and cytosine (5) and their semilogarithmic transformations. 1 and 5–52°, 2–62°, 3–72°, 4–79°

of DNA decomposition. It was expedient to verify whether the character of the luminescence really reflects the kinetics of the decomposition process of peroxide compounds of DNA.

In the present work, the rate of decomposition of peroxide compounds of DNA was studied by chemical methods, and data were obtained indicating agreement between the kinetic characteristics of the decomposition of peroxide compounds of DNA and the luminescence of irradiated DNA solutions.

In the work, 0.1% aqueous solutions of a native DNA preparation isolated from rat spleen by the Zubay and Doty method with deproteinization according to Kay ([4]) were used. DNA solutions were irradiated with X-rays on a RUT-200-20-3 installation at a dose of 70 kr in open thermostatted glass cells at 22°. Peroxide compounds in the irradiated DNA solution were analyzed spectrophotometrically ([5]). The hydroperoxide concentration was determined from the difference between the concentrations of peroxide compounds and hydrogen peroxide.

Fig. 3. Kinetic curves of hydroperoxide decomposition in irradiated solutions of thymine (1-4) and cytosine (5) and their semilogarithmic transformations. 1 and 5–52°, 2–62°, 3–72°, 4–79°.

Figure 1a presents the kinetic curves of decomposition of DNA hydroperoxide compounds at temperatures of 50-80°. Since it could be assumed that the decomposition of individual hydroperoxide compounds formed from pyrimidine bases would obey the kinetic law of first-order reactions, we constructed semilogarithmic transformations for all kinetic curves. One such transformation (for 52°) is shown in Fig. 1b. It is evident that the kinetic curves do not become completely linear in semilogarithmic coordinates. This could be associated with the occurrence in the system of two first-order processes having different rates. It was natural to suppose that we were dealing with the decomposition of two hydroperoxides differing in their stability. From the semilogarithmic transformations, the values of the rate constants for decomposition of the more stable hydroperoxide (k_2) were calculated from the slope of the linear portion. By extrapolating the linear portion of the transformation to zero, the values of the concentration of the more stable hydroperoxide (C_2) at the initial moments of time were calculated. By subtracting the C_2 values obtained in this way from the values of the total hydroperoxide concentration at the beginning of the decomposition process, the kinetic curve of decomposition of the less stable hy-

Fig. 4. Semilogarithmic anamorphosis of the kinetic curve of the change in viscosity of an irradiated DNA solution at 22°. Irradiation dose 70 kg

Figure 3: Fig. 4. Semilogarithmic anamorphosis of the kinetic curve of the change in viscosity of an irradiated DNA solution at 22°. Irradiation dose 70 kg

droperoxide was obtained; it gives a good linear dependence in the coordinates $\lg C$—time (Fig. 1c). From the slope of such linear transformations, the values of the rate constants for decomposition of the less stable hydroperoxide k_1 were calculated (see Table 1). As is evident from the table, the values of k_1 differ by an order of magnitude from the corresponding values of k_2. From the constant values, the activation energies of decomposition of DNA hydroperoxides were calculated:

Table 1

	52°	62°	72°	79°	90°
k_1, min^{-1}	$5.5 \cdot 10^{-2}$	$7.8 \cdot 10^{-2}$	$1.2 \cdot 10^{-1}$	$2.3 \cdot 10^{-1}$	$2.8 \cdot 10^{-1}$
k_2, min^{-1}	$2.7 \cdot 10^{-3}$	$5.8 \cdot 10^{-3}$	$1.4 \cdot 10^{-2}$	$6.6 \cdot 10^{-2}$	$3.6 \cdot 10^{-2}$
k of thymine, min^{-1}	$3.8 \cdot 10^{-3}$	$9.2 \cdot 10^{-3}$	$3.2 \cdot 10^{-2}$	$6.9 \cdot 10^{-2}$	

$E_1 = 13$ kcal/mole, $E_2 = 24$ kcal/mole. The Arrhenius dependence of k_1 and k_2 on temperature is presented in Fig. 2.

We also investigated the rate of decomposition of hydroperoxides in irradiated 0.1% solutions of thymine and cytosine. Fig. 3 presents the kinetic curves for the decomposition of thymine hydroperoxide at temperatures of 52-80° and of cytosine hydroperoxide at 52°. The kinetic curves are well straightened in semilogarithmic coordinates. From the slope of these anamorphoses, the values of the rate constants listed in Table 1 were calculated. Cytosine hydroperoxide decomposes at a substantially higher rate than thymine hydroperoxide at the same temperature: at 52°, $5.1 \cdot 10^{-1}$ and $3.8 \cdot 10^{-3}$ min^{-1}, respectively.

Fig. 4. Semilogarithmic anamorphosis of the kinetic curve of the change in viscosity of an irradiated DNA solution at 22°. Irradiation dose 70 kg.

The values of the rate constants for the decomposition of thymine hydroperoxide proved to be close to the corresponding values of the rate constants for the decomposition of the more stable DNA hydroperoxide, and the activation energies coincided (see Fig. 2).

Thus, the kinetic data make it possible to assume that the more stable hydroperoxide in irradiated DNA solutions is thymine hydroperoxide. The less

stable hydroperoxide, apparently, may be cytosine hydroperoxide, which, however, requires further study.

Table 2

	k_1, min^{-1}	k_1, min^{-1}	k_2, min^{-1}	k_2, min^{-1}
	HP	viscosity	HP	viscosity
Our data	$1 \cdot 10^{-2}$	$0.9 \cdot 10^{-2}$	$1.1 \cdot 10^{-4}$	$2.9 \cdot 10^{-4}$
Weiss' s data	$1 \cdot 10^{-2}$	$0.6 \cdot 10^{-2}$	$4.0 \cdot 10^{-4}$	$4.8 \cdot 10^{-4}$

The value of the activation energy for the decomposition of the unstable hydroperoxide in irradiated DNA solutions ($E = 13$ kcal/mole) agrees well with the value ($E = 11 \pm 2$ kcal/mole) found from chemiluminescence data. Such agreement supports the assumption that the decomposition of DNA hydroperoxide compounds may be the cause of the appearance of luminescence.

The results obtained by us make it possible to put forward certain assumptions concerning the possible mechanism of the phenomenon of radiation aftereffect observed in irradiated DNA solutions. Some authors have indicated the possible participation of peroxide compounds in this phenomenon (6). Our data show that the kinetic curves of the change in viscosity of irradiated DNA solutions are analogous to the curves of decomposition of peroxide compounds-

of DNA compounds. By representing the curve of the change in viscosity in semilogarithmic coordinates, one can calculate the values of the constants k_1 and k_2, in the same way as was done for the decomposition of the peroxide compounds of DNA (Fig. 4). The values of the constants found were compared with values calculated in an analogous manner from our data for the decomposition of DNA hydroperoxides, and also from the data of Weiss ([7]) for a temperature of 22° (Table 2).

As can be seen from Table 2, the values of the constants from the data on the change in viscosity and on the decomposition of hydroperoxides agree well with one another, which may indicate the substantial role of hydroperoxide compounds in the phenomenon of radiation aftereffect observed in irradiated DNA solutions.

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REFERENCES CITED

[1] G. Scholes, J. Weiss, C. M. Wheeler, Nature, **178**, 157 (1956).
[2] R. Latarjet, B. Ekert, P. Demersmen, Radiation Res., Suppl. **3**, 247 (1963).

[3] N. M. Emanuel' , K. E. Kruglyakova et al., Izv. AN SSSR, OKhN, 1963, No. 6, 1143.
[4] G. Zubay, P. Doty, J. Mol. Biol., **1**, 1 (1959); E. R. M. Kay, N. S. Simmons, A. H. Dounce, J. Am. Chem. Soc., **74**, No. 7, 1724 (1952).
[5] J. Hochenadél, J. Phys. Chem., **56**, 587 (1952); G. N. Eisenberg, Ind. Eng. Chem. Anal. Ed., **15**, 327 (1943).
[6] J. A. V. Butler, *Organic Peroxide in Radiobiology*, Masson, Paris, 1958.
[7] M. Daniels, G. Scholes et al., J. Chem. Soc., 1957, No. 1, 226.

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