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Abstract

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ON THE MECHANISM OF RECOMBINATION OF RADICALS OF IRRADIATED PROTEINS IN THE PRESENCE OF OXYGEN

A considerable number of works have been devoted to the study of recombination of protein radicals. The principal experimental data on the effect of oxygen on recombination amount to the following:

a. When oxygen is admitted into ampoules containing a protein irradiated in vacuum, the appearance of the EPR signal changes—the unresolved doublet ($R_0^{\bullet}$) is converted into a singlet ($R_1^{\bullet}$) ([1,2]).

b. The lifetime of radicals varies substantially for different proteins (from minutes for hemoglobin radicals to hours for radicals of irradiated casein) and is determined by the penetration of oxygen into the sample. The kinetic curves for the decay of radicals are usually described by equations of first- or second-order reactions ([1,3]).

c. The effective activation energies are 8–12 kcal/mole ([3]).

d. The death of radicals in vacuum is strongly hindered (the disappearance times of EPR signals reach several months).

e. EPR spectra of peroxide radicals of proteins have been detected at low temperatures ([4]).

f. On contact of oxygen with an irradiated protein, chemiluminescence arises, caused by disproportionation of peroxide radicals ([3,5]).

On the basis of these data one may imagine the following schematic sequence of alternation of radicals during recombination:

$$R_0^{\bullet} \xrightarrow{O_2;\ \text{water}} R_1^{\bullet} \xrightarrow{O_2} RO_2^{\bullet} \rightarrow \text{nonradical products.}$$

At room temperature the peroxide radicals of irradiated proteins are not stabilized; their stationary concentration is small and their registration by the EPR method is difficult.

The kinetic parameters of radical decay in dry proteins are very similar to the parameters of radical death in irradiated synthetic polymers ([6]). It was therefore natural to suppose that the process of death of radicals of irradiated proteins (as also for the recombination of radicals in polymers ([7—9])) may include a stage of transfer of free valence, i.e., proceed by the mechanism of chain oxidative recombination. In this case the proposed recombination mechanism will have the following form:

$$R_0^{\bullet} + O_2 \rightarrow R_0O_2^{\bullet} \quad \text{(I)};$$

$$R_0O_2^{\bullet} + R_1H \rightarrow R_0OOH + R_1^{\bullet} \quad \left(\text{or } R_0^{\bullet} \xrightarrow{O_2;\ \text{water}} R_1^{\bullet}\right) \quad \text{(II)};$$

$$R_1^{\bullet} + O_2 \rightarrow R_1O_2^{\bullet} \quad \text{(III)};$$

$$R_1O_2^{\bullet} + R_1H \rightarrow R_1OOH + R_1^{\bullet} \text{ etc.} \quad \text{(IV)};$$

$$2R_{01}O_2 \rightarrow \text{nonradical products} \quad \text{(V)}.$$

Using the EPR method, the occurrence of reactions forming peroxide radicals (I and III) ([4]), the existence of the radical R_1, different from the initial R_0 ([1,2]), and the presence of process V (this has also been confirmed by the chemiluminescence method) ([5]) have been shown.

A direct indication that processes proceed according to the scheme of chain oxidation may be provided by data on the magnitudes of oxygen absorption during oxidative recombination of radicals.

If the process includes only stages 1 and 5, then 0.5 molecule of oxygen should be consumed per radical. If, in addition, processes 2 and 4 occur, then the amount of absorbed oxygen should be greater than 0.5 molecule/radical.

In the present work, a study was made of oxygen absorption by irradiated proteins and of radical decay under comparable conditions. Two proteins were used—casein and bovine serum albumin.

The initial number of radicals and the kinetics of their decay were measured on an EPR-2 radiospectrometer of the Institute of Chemical Physics. Absolute measurements of the number of radicals were carried out by comparing the EPR spectra of irradiated proteins with the spectra of single crystals of copper chloride. The single crystals were weighed on analytical microbalances.

The number of radicals in protein samples was determined by comparing the values of the double integrals of the derivative absorption band of the standard and of the sample being measured.

The reproducibility of the measurement results was 10–15%; the overall accuracy of the absolute measurements did not exceed 20–30%.

The amount of absorbed oxygen was measured in a capillary microrespirometer ([10]) placed in a thermostated vessel ($\pm 0.01^{\circ}$). The microrespirometer consisted of an ampoule with a ground joint and a long capillary, into which a drop of kerosene was placed. The capillaries of the respirometers were calibrated beforehand.

The readings of the drop movement were recorded semi-automatically on an EPP-09 potentiometer by means of a simple potentiometric tracking system. With the apparatus it was possible to measure a change in the position of the drop upon absorption of 1 mm^3 of gas over 10–20 min. Oxygen absorption measurements were carried out at a temperature of 22°, 30 sec after admission of the gas into the microrespirometer. The duration of the experiments reached one day—for determination of complete absorption.

Crystalline proteins were irradiated on a GUT-Co-400 gamma installation with a dose of 20–50 Mrad in sealed microrespirometers and ampoules made of glass that did not give EPR signals upon irradiation. The respirometers and ampoules were evacuated before irradiation with a fore-vacuum pump for 10–15 min.

In the very first experiments, intense absorption of oxygen by the irradiated proteins was recorded, continuing for several hours. However, before obtaining quantitative data, it was necessary to make sure that oxygen is absorbed as a result of reactions with radicals, and to determine the time parameters for filling the samples with gas.

For this purpose the following experiments were carried out:
1. A respirometer with nonirradiated protein was evacuated and then the absorption of gas (air and argon) was measured under the experimental conditions. 2. The absorption of argon by irradiated protein was measured. 3. The absorption of gas (air and argon) by protein after disappearance of all radicals was measured.

It turned out that filling the protein samples with gas is completed within 8–12 min. The total amount of absorbed gas is determined by the diffusion parameters and by its solubility in the samples. The absorption of argon exceeds the absorption of air. The amount of absorbed gas in all three series of experiments was the same and amounted to 5–10% of the total absorption of oxygen by the irradiated protein. These corrections were introduced into the measurement results.

Next, radicals in evacuated ampoules were eliminated by heating at 100° for 1 hour, after which oxygen absorption was absent. In addition, water was added to ampoules containing protein irradiated in vacuum; the radicals decayed within 5–10 min, and the water was then evacuated. In this case no additional oxygen absorption was observed.

As a result of the series of control experiments described, it could be considered

Fig. 1. Kinetic curves of oxygen absorption (a) and radical decrease (b) in irradiated casein (I) and bovine serum albumin (II)

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that oxygen absorption (after subtracting the amount used for filling the sample) is associated with reactions of radicals in the irradiated protein.

In Fig. 1, *I*, typical curves are presented for oxygen absorption (*a*) and radical decay (*b*) in irradiated casein. In Fig. 1, *II*—the same curves for irradiated serum albumin. The values of the amount

absorbed oxygen and the number of radicals were referred to 1 g of protein. It is seen that after admission of air into the system the number of radicals decreases and oxygen is absorbed. The rates of these processes are greatest at the beginning and gradually decrease. The total time for the decrease of radicals and absorption of oxygen was 15-20 h.

The amount of oxygen absorbed is greater than the number of radicals recombined during this time. From this one can calculate the "chain length" ν, i.e., the ratio of the mean rates of oxygen absorption to the rate of radical decrease; this was found to be, for serum albumin, 2.9-3.5 molecules of O_2 per radical, and for casein, 1.8-28.

Fig. 1. Kinetic curves of oxygen absorption (**a**) and radical decrease (**b**) in irradiated casein (**I**) and bovine serum albumin (**II**)

It should, of course, be taken into account that the values of ν are unlikely to be determined with an accuracy better than 30%. Nevertheless, even in this case the values of the "chain length" substantially exceed 0.5—the theoretical number of oxygen molecules calculated per one radical.

Of special interest are the values of ν at low radical concentrations, when the distances between them are large. However, obtaining reliable data is associated with considerable experimental inaccuracies. In one of the preliminary experiments a value of ν exceeding 7 was obtained for irradiated casein when the radical concentration was less than 0.1×10^{-6} mol/g, but the accuracy of the measurement in this case is low.

Thus, it may be considered that recombination of radicals of irradiated proteins in the presence of oxygen can proceed by the mechanism of chain oxidative recombination.

Chemical analysis of the products of protein radiolysis does not contradict the possible recombination scheme (I-V). Peroxide compounds were found ([11]), which can be formed in the chain-transfer reactions II and IV. An increase in the content of carbonyl groups was detected ([12]), which can be formed in the disproportionation reaction of peroxide radicals V. For a final solution of the

question of the possibility of chain oxidative recombination, quantitative data are needed on the yields of peroxides, carbonyls, and oxy groups calculated per one radical of the irradiated protein.

It may be hoped that further studies will make it possible to approach the question of the possibility of chain oxidative recombination processes in more complex structures and fragments of cells and to relate the radiobiological oxygen effect to reactions of this kind.

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