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Mechanisms of Initiated Protein Peroxidation Accompanied by Generation of Carbonyl Fragments

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Abstract — Regularities of the peroxide fragmantation of human serum albumin, initiated by an iron(II) salt and hydrogen peroxide, are considered. A chain mechanism is proposed for this process, leading to generation of carbonyl fragments of the protein molecule.

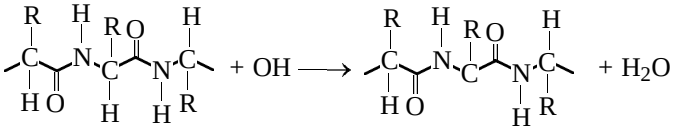
Whereas protein peroxidation processes at various pathological states [1–10] and effects of chemical substances on living bodies [11–15] have been widely studied, no serious systematic attempts to characterize the fragmentation of the polypeptide chain as such and to propose a mechanism of this reaction have been undertaken.

Initiated protein peroxidation can arbitrary be divided into two steps: generation of the main species initiating damage of the polypeptide chain ($^{\cdot}OH$); attack of the $^{\cdot}OH$ radical on the polypeptide chain and development of the process in two directions: cleavage of the polypeptide chain (fragmentation) and generation of smaller fragments containing carbonyl groups {it is these carbonyl fragments which are determined in medical and biological practice as the products of peroxide protein damage (as adducts with 2,4-dinitrophenylhydrazine [16–19]}; formation of $RO^{\cdot}$ radicals from hydroxyamino acids (primarily tyrosine) and of $RS^{\cdot}$ radicals from thioamino acids, followed by aggregation of the resulting species.

The process can be chemically initiated by a mixture of hydrogen peroxide with copper(I) and iron(II) salts, organic peroxides capable of homolytic cleavage, mixtures of organic hydroperoxides with copper(I) and iron(II) salts, a mixture of nitrite ion with hydrogen peroxide or organic hydroperoxides, mixtures of various thiols with hydrogen peroxide, and some other compositions. The most frequently used initiator is a mixture of iron(II) salts with hydrogen peroxide, which has become a classical system [20–24].

It the present work we made an attempt to examine in detail the fragmentation of the polypeptide chain.

We suggest that the most vulnerable fragment of the polypeptide chain is an α-methine group of the protein molecule, which is in the periphery of the protein helix [25–27]. The most probable initial fragmentation steps are represented below [schemes (1)–(3)].



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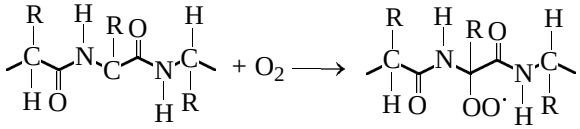

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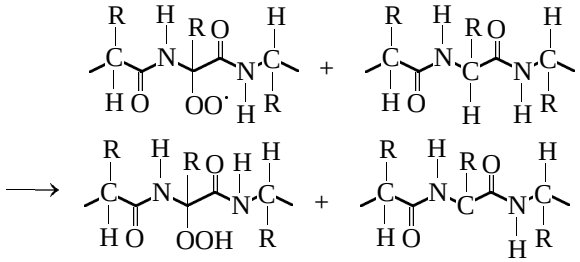

(3)

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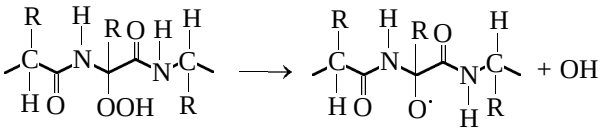
Regeneration of the –·CR– radical and hydroperoxide formation are the most probable steps of this process.

Particular attention should further be paid to the fate of the resulting hydroperoxide. The latter can undergo homolytic cleavage [reaction (4)] followed by decomposition of the hydroxyl radical by reactions (5) and (6) and generation of carbonyl fragments which are final products of the peroxidation process.



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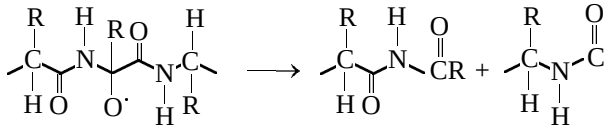

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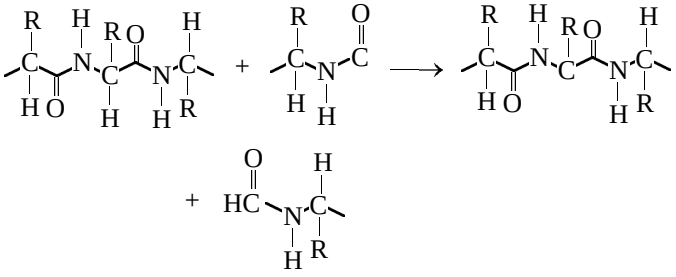
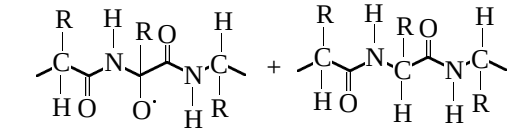


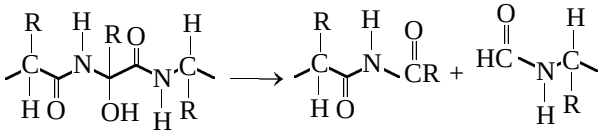
(6)

However, the hydroxyl radical can also actively react with the native protein [scheme (7)].

The hydroxy derivatives thus formed can decompose to give carbonyl fragments [scheme (8)].



(7)



(8)

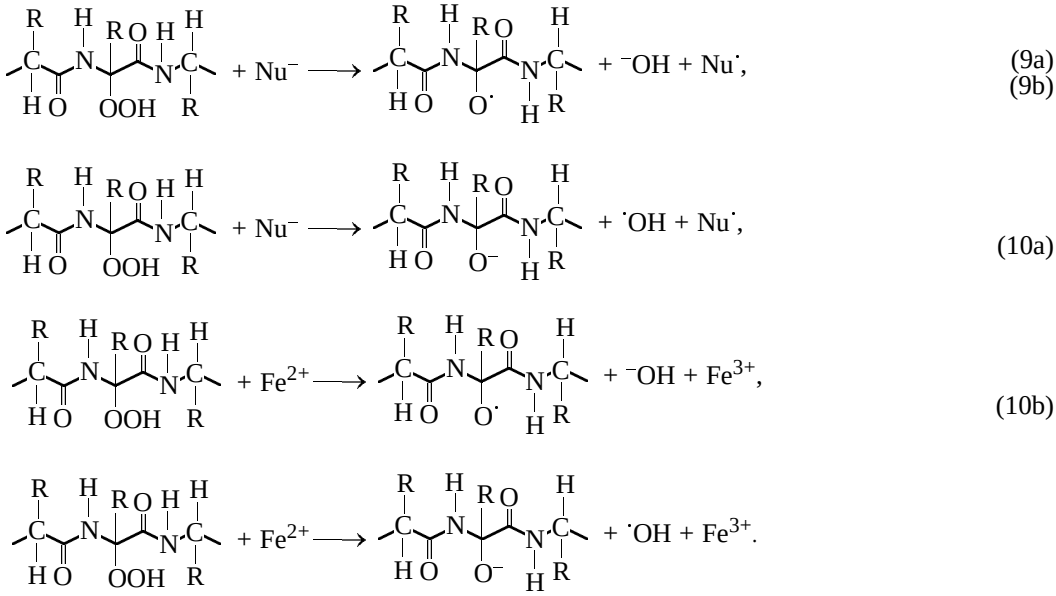
It should be noted that the most probable in aqueous medium in a nucleophilic attack on the resulting peroxide [scheme (9)].

The role of nucleophiles can be played by ionized fragments of the protein molecule, such as –S^-–, –O^-–, and –NH–CR^-–CO^-.

The resulting hydroxyl radical and alkoxide anion are unstable and can decompose to give carbonyl

compounds, as described above.

In the case of the classical hydrogen peroxide–iron(II) salt initiation of protein peroxidation, the probability of heterolytic cleavage of the hydroperoxide formed is especially high [scheme (10)].

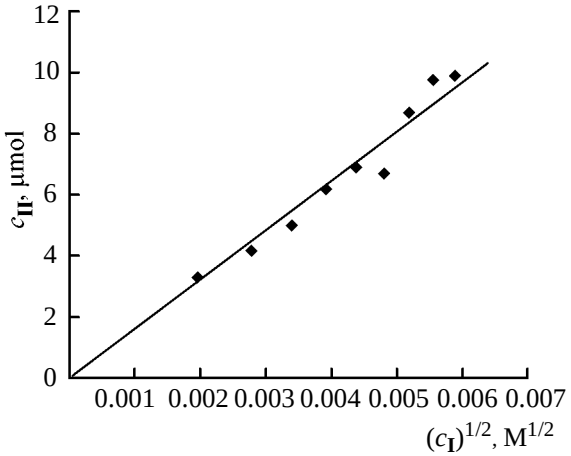


Thus, in view of the aforesaid, peroxide protein fragmentation can be considered as a radical chain process. Therewith, the question of the stage of chain termination remains open. It can be proposed that chain termination is effected by recombination of hydroxyl radicals, but this is questionable.

Another possible way to chain termination is recombination of two hydroxyl radicals or of one hydroxyl radical and the primary polypeptide radical. Such reactions give rise to protein aggregates. This is not inconsistent with published data, since it is well known that peroxide protein damage is accompanied by aggregation processes [28–31].

To elucidate the mechanism of chain termination, one should find out relation between the accumulation of peroxidation products and the protein concentration.

We found that the intensity of initiated peroxide damage of human serum albumin (**I**) is proportional to the square root of the protein concentration (see figure).

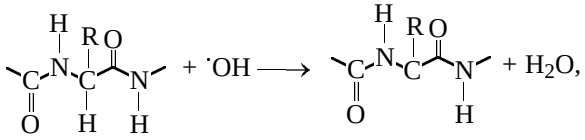


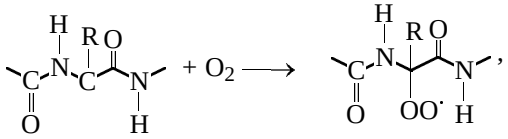
Quantity of carbonyl protein fragments determined as 2,4-dinitrophenylhydrazones (c_{II}, μM) vs. square root of albumin concentration (c_I, $M^{1/2}$). Aqueous phosphate buffer, pH 7.4, $c_{H_2O_2}$ 4.21×10^{-2} M, $c_{Fe^{2+}}$ 1.84×10^{-4} M, $c_{albumin}$ 3.85–34.62 μM.

In terms of the classical theory of chain processes, with chain initiation by reaction (11), the dependence of the accumulation of carbonyl compounds on the albumin concentration (see figure) can be related to chain unbranched processes with cross chain termination. This process can be described by schemes (12)–(18), where stage (11) is chain initiation, stages (12)–(17) are chain propagation, and stage (18) is chain termination.

As seen from the schemes, the limiting stage of the peroxide damage does not involve two or more charged species. This is indirectly supported by the

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\cdot}OH + {}^{-}OH \quad (11)$$


(12)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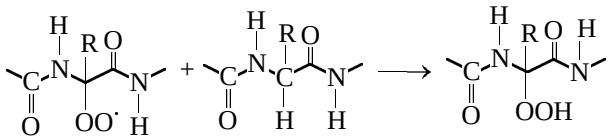
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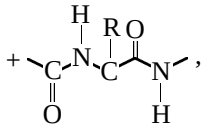
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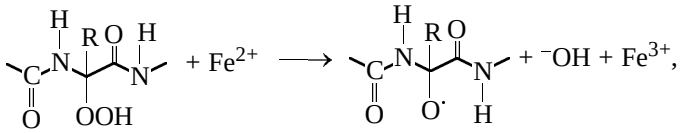
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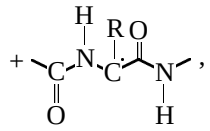
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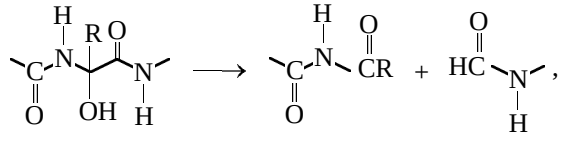
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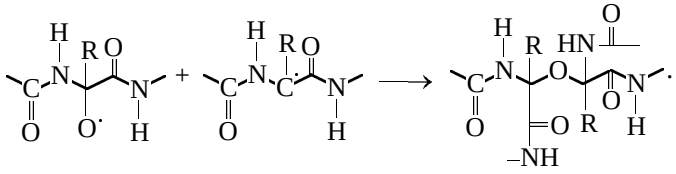

experimental data which show that in a phosphate buffer (pH 7.4) and in physiological solution the intensities of peroxidation are similar, whereas the ionic strengths of the solutions are different: 0.52 and 0.17 M, respectively [32].

Furthermore, the reaction involves no protolytically active species, which agrees with the fact that the intensity of accumuation of the reaction products determined as 2,4-dinitrophenylhydrazones **II** is independent of the pH of the medium (in the range 5.8–8.0).

By the theory of chain processes, the rate v of a process with cross chain termination is determined by expressions (19) and (20) [33–37]:

$$v = a(v_0)^{1/2}, \quad (19)$$

$$v_0 = k_1 c_{H_2O_2} c_{Fe^{2+}}. \quad (20)$$

Here v is the reaction rate, v_0 is the initiation rate, a is the proportionality coefficient, and k_1 is the initiation rate constant.

The rate v is proportional to the square root of the concentrations of reactants that react with radicals participating in chain termination. In this case, with the limiting stages (13) and (16), the most probable is expression (21):

$$v = b(c_{\mathbf{I}} c_{O_2})^{1/2}. \quad (21)$$

Here b is the proportionality coefficient. The occurrence of such dependence with albumin we confirmed experimentally (see figure). Elucidation of the relation between the intensity of the process and the concentration of oxygen is an intricate problem, since the latter concentration is difficult to maintain constant and to measure.

EXPERIMENTAL

A phosphate buffer (pH 7.4) was added to a freshly prepared 1% solution of freeze-dried human serum albumin in twice distilled water so that the volume of the solution was 0.8 ml, after which a fresly prepared mixture of 0.1 ml of 0.06% aqueous iron(II) sulfate and 0.06% aqueous EDTA (1 : 1) and 0.1 ml of 15% hydrogen peroxide was added. Incubation was performed on a water bath at 37°C for 15 min.

The process of albumin peroxide oxidation was terminated with 1 ml of 20% trichloroacetic acid. The mixture was treated with 1 ml of a 0.2% solution of 2,4-dinitrophenylhydrazine in 2 N HCl to convert the resulting carbonyl fragments to the colored 2,4-dinitrophenylhydrazones. The reaction mixture was left to stand at room temperature for 1 h.

The precipitate that formed was separated by centrifugation (15 min at 3000 rpm), treated with 3 ml of a freshly prepared mixture of ethanol and ethyl acetate (1 : 1, by volume) to remove excess 2,4-dinitrophenylhydrazine, separated by centrifugation (15 min at 3000 rpm), and dried. The dry precipitate was treated with 5 ml of 8 M aqueous urea, and the mixture, after addition of 1–2 drops of 2 N HCl (for better dissolution), was heated on a water bath at 37°C until the precipitate dissolved completely.

The intensity of the polypeptide chain damage was followed spectrophotometrically on an SF-46 specrtophotometer at a wavelength of 363 nm by the accumulation of colored hydrazones.

The concentrations of the hydrazones were estimated with an extinction coefficient ε of 2.2×10^4 l mol^{-1} cm^{-1} [18].

REFERENCES

1. Fridovich, I., *Science*, 1978, vol. 201, no. 4359, pp. 875–880.
2. Men'shikova, E.B., Zenkov, N.K., and Safina, A.F., *Usp. Sovrem. Biol.*, 1997, vol. 117, no. 3, pp. 362–373.
3. Men'shikova, E.B. and Zenkov, N.K., *Usp. Sovrem. Biol.*, 1997, vol. 117, no. 1, pp. 155–171.
4. Wolf, S.P., Garner, A., and Dean, R.T., *Trends Biochem. Sci.*, 1986, vol. 11, no. 1, pp. 27–31.
5. Das, D., Bandyopadhyay, D., and Banerjee, R.K., *Free Rad. Biol. Med.*, 1998, vol. 24, no. 3, pp. 460–469.
6. Langvist, H. and Dahlgren, C., *Free Rad. Biol. Med.*, 1996, vol. 20, no. 6, pp. 785–792.
7. Srigiridhar, K. and Modhavan Nair, K., *Free Rad. Biol. Med.*, 1998, vol. 25, no. 6, pp. 660–665.
8. Kotch, L.E., Chen, S.Y., and Sulik, K.K., *Teratology*, 1995, vol. 52, no. 2, pp. 128–136.
9. Brown, K.E. and Knudsen, C.A., *Free Rad. Biol. Med.*, 1998, vol. 24, no. 2, pp. 239–244.
10. *Natural Antioxidants in Human Health and Disease*, Frei, B., Ed., New York: Academic, 1994.
11. Ischiropoulos, H. and Al-Meholi, A.B., *FEBS Lett.*, 1995, vol. 364, no. 3, pp. 279–282.
12. Gatti, R.M., Radi, R., and Augusto, O., *FEBS Lett.*, 1994, vol. 348, no. 3, pp. 287–290.
13. Ereal, N., Treeratphan, P., Hammond, T.C., Matthews, R.H., Grannemann, N.H., and Spits, D.K., *Free Rad. Biol. Med.*, 1996, vol. 21, no. 2, pp. 157–161.
14. Ketterer, B., Loles, B., and Meyer, D.J., *Environ. Health. Perspect.*, 1983, vol. 49, no. 1, pp. 59–69.
15. Hsu, J.M., *J. Nutrit.*, 1981, vol. 111, no. 1, pp. 26–33.
16. Reznik, A.Z. and Packer, L., *Methods Enzymol. C*, 1994, vol. 233, pp. 357–363.
17. Lee, Y. and Schacher, E., *Arch. Biochem. Biophys.*, 1995, vol. 321, no. 1, pp. 175–181.
18. Reinkeckel, T., Medelev, B., Prause J. Augustin, W., Schulz, H.U., Lippert, H., and Halangk, W., *Free Rad. Biol. Med.*, 1998, vol. 24, no. 3, pp. 393–400.
19. Levine, R.L., Williams, J.A., Stadtman, E.R., and Schacher, E., *Methods Enzymol. C*, 1994, vol. 233, pp. 346–357.
20. Zenkov, N.K. and Men'shikova, E.B., *Usp. Sovrem. Biol.*, 1996, vol. 116, no. 6, pp. 729–748.
21. Reszka, K.J., Matuszak, L., and Chignell, C.F., *Free Rad. Biol. Med.*, 1998, vol. 25, no. 2, pp. 208–216.
22. Halliwell, B. and Gutteridge, J.M.C., *Arch. Biochem.*

Biophys., 1986, vol. 246, no. 2, pp. 501–514.

23. Winston, G.W., Regoli, F., Dugas, A.J., Fong, J.H., and Blanchard, K.A., *Free Rad. Biol. Med.*, 1998, vol. 24, no. 3, pp. 480–493.
24. Halliwell, B. and Gutteridge, J.M.C., *Methods Enzymol.*, 1990, vol. 186, no. 1, pp. 1–85.
25. Stroev, E.A., *Biologicheskaya khimiya* (Biological Chemistry), Moscow: Vysshaya Shkola, 1986.
26. Tyukavina, N.A. and Baukov, Yu.I., *Bioorganicheskaya khimiya* (Bioorganic Chemistry), Moscow: Meditsina, 1985.
27. Shulz, G. and Schirmer, R., *Principles of Protein Structure*, New York: Springer, 1979.
28. Shikama, K., *Chem. Rev.*, 1998, vol. 98, no. 4, pp. 1357–1373.
29. Stubbe, J. and Donk, W.A. van der, *Chem. Rev.*, 1998, vol. 98, no. 2, pp. 705–762.
30. Dubinina, E.E. and Shugalei, I.V., *Usp. Sovrem. Biol.*, 1993, vol. 113, no. 1, pp. 71–81.
31. Igumenov, V.L., Sharonov, B.P., and Pasechnikov, V.A., *Biokhimiya*, 1988, vol. 53, no. 6, pp. 925–930.
32. Pal'm, V.A., *Osnovy kolichestvennoi teorii organicheskikh reaktsii* (Principles of Quantitative Theory of Organic Reactions), Leningrad: Khimiya, 1977.
33. Emanuel', N.M. and Evseenko, L.S., *Kolichestvennye osnovy klinicheskoi onkologii* (Quantitative Principles of Clinical Oncology), Moscow: Meditsina, 1970.
34. Emanuel', N.M., Denisov, E.T., and Maizus, Z.K., *Tsepnye reaktsii okisleniya uglevodorodov v zhidkoi faze* (Chain Reactions of Liquid-Phase Oxidation of Hydrocarbons), Moscow: Nauka, 1965.
35. Emanuel', N.M., Zaikov, G.E., and Maizus, Z.K., *Rol' sredy v radikal'no-tsepnykh reaktsiyakh okisleniya organicheskikh soedinenii* (Role of Medium in Radical–Chain Oxidation Reactions of Organic Reactions), Moscow: Nauka, 1973.
36. Emanuel', N.M. and Knorre, D.G., *Kurs khimicheskoi kinetiki* (Handbook on Chemical Kinetics), Moscow: Vysshaya Shkola, 1974.
37. Emanuel', N.M., Zaikov, G.E., and Kritsman, V.A., *Tsepnye reaktsii. Istoricheskii aspekt* (Chain Reactions. A Historical Aspect), Moscow: Nauka, 1989.