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Peroxide Damage of Hemoglobin by the Fenton System

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Abstract — The extent of hemoglobin peroxidation under the action of mixtures of ferrous salts and hydrogen peroxide has been investigated. The rate of accumulation of carbonyl-containing products of the protein fragmentation, detected as 2,4-dinitrophenylhydrazones, is independent of the pH of the reaction medium and proportional to the concentrations of hydrogen peroxide, ferrous ion, and hemoglobin. A chain radical mechanism of the peroxide fragmentation of the polypeptide chains of hemoglobin has been proposed, involving reactions of the alkoxyl radical of hemoglobin with ferrous ion and of the carbon-centered radical of the protein with dissolved oxygen as rate-limiting steps. Chain termination is therewith effected through cross recombination of the above radicals.

In view of the fact that the key role in peroxide protein damage most frequently belongs to hydroxyl radical [1–3], of particular interest is to study the peroxide fragmentation of polypeptide chains of different proteins under the action of a mixture of ferrous salts with hydrogen peroxide (Fenton system), which readily generates $OH^{\cdot}$ radical [4]. Hemoglobin in this connection deserves close attention, because, along with the polypeptide chains, the active intermediates of the Fenton system are capable of damaging the prostetic group of this protein [5–7].

It is a common knowledge that hydrogen peroxide facilitates oxidation of the iron atom in the prostetic group of hemoglobin [6, 7], generating methemoglobin. The oxidation is a chain process which has been thoroughly studied [4–8]. The ferrous ion also promotes methemoglobin production [6]. This effect is assigned to late-stage catalysis of autooxidation produced by peroxides accumulated in the reaction system.

Taking into account the above-mentioned facts, we considered it of great interest to focus on the possible damage of the polypeptide chains of this protein under conditions of active generation of hydroxyl radicals [reaction (1)]:

$$Fe^{2+} + H_2O_2 \rightleftarrows Fe^{3+} + OH^- + OH^{\cdot} \qquad (1)$$

Hemoglobin, owing to the presence of the hemine group bearing ligated oxygen, is in itself capable of generating active oxygen species, which seriously complicates the process. Radical centers can be generated in a several independent ways [reactions (1)–(3)]:

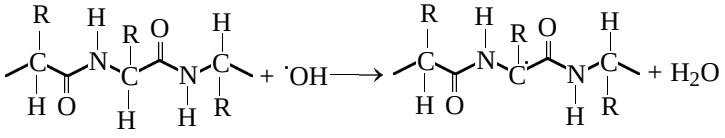
$$HbO_2(Fe^{2+}) + H^+ \rightleftarrows MetHb(Fe^{3+}) + HO_2^-, \qquad (2)$$

$$HbO_2(Fe^{2+}) + H_2O_2 \rightleftarrows MetHb(Fe^{3+}) + OH^- + OH^{\cdot} + O_2. \qquad (3)$$

Basing on published data [8], the contribution of reaction (2) can be neglected. Then the generation of radical species can be described by the overall reaction (4):

$$Fe^{2+} + 2H_2O_2 + HbO_2(Fe^{2+}) \rightleftarrows MetHb(Fe^{3+}) + 2OH^- + 2OH^{\cdot} + O_2. \qquad (4)$$

This process is most probably developed by a chain mechanism, similarly to peroxidation of other proteins [9, 10]. Hydroxyl radical is the most active species among all generated radicals [6, 11] and should be preferred as a chain-leading radical. As with simple proteins (for example, albumin [9, 10]), in is hydroxyl radical that attacks the polypeptide chain according to reactions (5)–(7):



(5)

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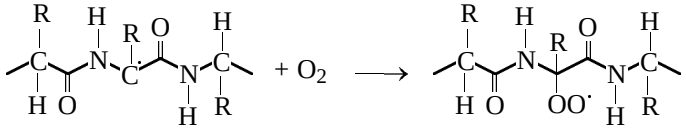

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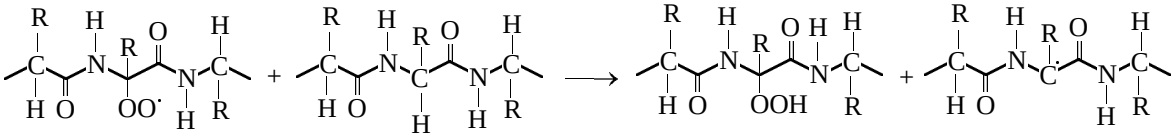
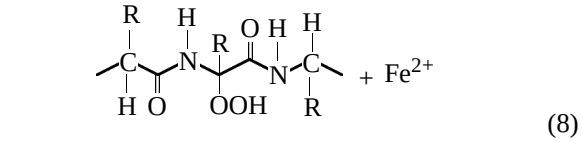


(7)

The ferrous ion present in the system considerably complicates further development of the process by reacting with the protein hydroperoxide formed [reactions (8) and (9)].



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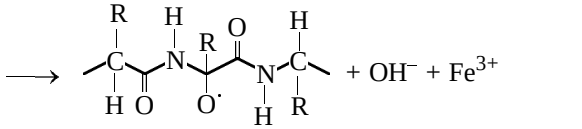
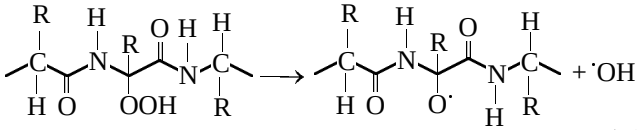


(9)

The protein hydroperoxide generated in the course of the process is also capable of fragmentation with subsequent formation of carbonyl-containing species as final products of the peroxidation process [reactions (10)–(12)]:



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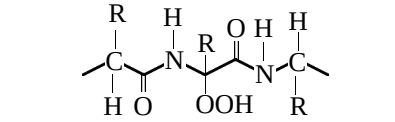
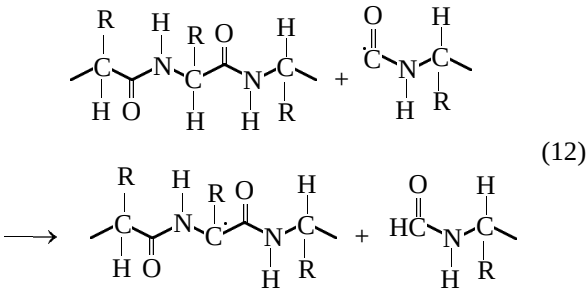
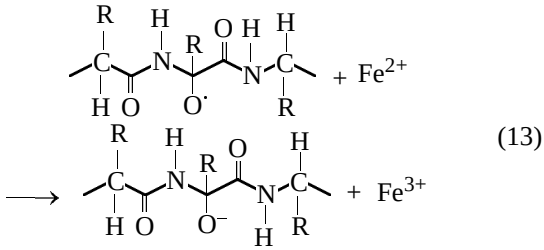


(11)

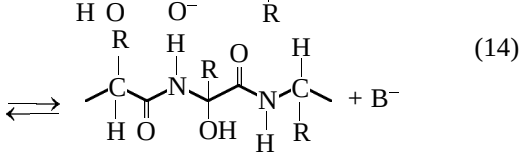


(12)

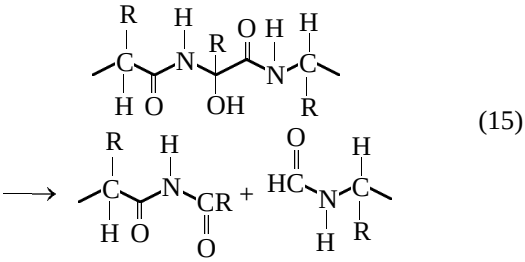
It should be mentioned that the alkoxyl radical generated in the course of the process can also interact with the ferrous ion present in the system [reaction (13)]. The alkoxide anion produced in this reaction generates, in buffer media, a hydroxy derivatives and further on carbonyl products of the protein fragmentation [reactions (14) and (15)].



(13)

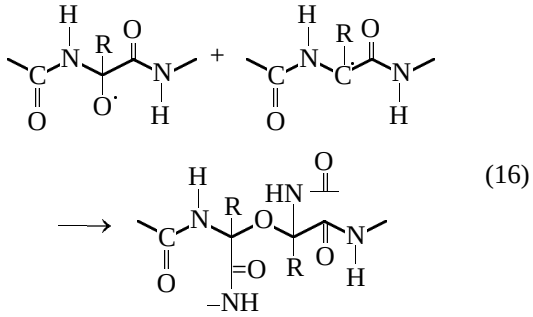


(14)



(15)

The most probable route for chain termination is radical recombination according to scheme (16):



(16)

The limiting stages of chain unit during this cross termination are reactions (6) and (13), and the chain unit involves the following stages: initiation stage (4), chain-propagation stages (5)–(7) and (10)–(15), and cross termination stage (16).

The validity of the above scheme can be supported by the results of study of reaction rate as a function of reagent concentrations.

According to scheme (4), the rate of initiation should follow Eq. (17):

$$v_0 = a[H_2O_2]^2[Fe^{2+}][HbO_2]. \quad (17)$$

In accordance with the regularities of chain processes with cross termination of chains [12], the reaction rate v is given by Eq. (18) or, on account of Eq. (17), by Eq. (19):

$$v = bv_0^{1/2}, \quad (18)$$

$$v = c[H_2O_2]([Fe^{2+}][HbO_2])^{1/2}, \quad (19)$$

$$c = ba^{1/2}. \quad (20)$$

In agreement with the proposed scheme, it is the ferrous ion and hemoglobin which take part in the rate-limiting stages of chain unit. It is well known [12] that the total rate of a process with cross termination of chains is proportional to the square root of the concentrations of the reagents that take part in the limiting stages of chain unit, as well as to the square root of the initiation rate. In this case, the resulting equation for the rate of accumulation of peroxidation products should have the form (21), which is in full agreement with the experimental results.

$$v = k[H_2O_2][Fe^{2+}][HbO_2]. \quad (21)$$

In Eqs. (17)–(21), a, b, c, and k are proportionality coefficients.

By a detailed study of the peroxide damage of hemoglobin, initiated by the Fenton system, we established major regularities of generation of carbonyl-containing products. The latter were detected as 2,4-dinitrophenylhydrazones (**I**) in aqueous phosphate buffer (pH 7.4). At the concentrations of hydrogen peroxide and ferrous ions of 42.1 and 0.18 mM, respectively, the concentration of peroxidation products varied with hemoglobin concentration according to Eq. (22):

$$c_I = 0.56c_{Hb}; \; r \; 0.943, \; n \; 9. \quad (22)$$

As the protein concentration was varied in the range 19.07–133.49 μM, the concentration of 2,4-dinitrophenylhydrazones varied from 6.23 to 73.55 μM.

At constant concentrations of ferrous ions (0.18 mM) and hemoglobin (31.01 μM) and the concentration of H_2O_2 varied in the range 0.02–0.32 M, the concentration of peroxidation products generated in the system varied according to Eq. (23).

$$c_I = 39.9c_{H_2O_2}; \; r \; 0.901, \; n \; 11. \quad (23)$$

In this case, the concentration of 2,4-dinitrophenylhydrazones varied from 1.86 to 13.73 μM.

At constant concentrations of H2O2 (42.1 mM) and hemoglobin (31.03 μM) and the concentration of ferrous ions varied from 0.92 to 11.04 mM, the concentration of the peroxide protein damage products varied according to Eq. (24):

$$c_I = 2.3c_{Fe^{2+}}; \; r \; 0.850, \; n \; 8. \quad (24)$$

In this case, the concentration of 2,4-dinitrophenylhydrazones varied from 0.09 to 27.09 μM.

The rate of the peroxidation process is shown to be independent of the pH of the reaction media, which supports the proposed reaction scheme of the peroxide protein damage, where proton is not involved into the limiting stages of chain unit.

Thus, the proposed scheme of the peroxide damage of the polypeptide chain of hemoglobin is in complete agreement with the established regularities of the process.

EXPERIMENTAL

Blood of healthy adults was used in the present work. Red cells were separated from serum and hemolizates were prepared according to the procedure reported in [13]. The concentration of hemoglobin in the resulting solutions was measured spectrophotometrically as described in [14]. The peroxide frag-

mentation of the polypeptide chains of hemoglobin was studied according to the procedure described in [10].

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