ROLE OF MYOGLOBIN TYROSINE RESIDUES IN THE DISPROPORTIONATION REACTION BETWEEN HEME IRON(II) AND HEME IRON(IV)

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The basic function of myoglobin [Mb(II)] namely to support the aerobic respiration of myocytes, depends on the ability of its heme groups to bind molecular oxygen reversibly. One of the main causes of loss of functional activity of Mb(II) in vivo is a change in the valency of the heme iron (Fe II) as a result of induction of redox processes [9, 10]. Hydrogen peroxide (H_2O_2), formed in the cell as a result of several biochemical reactions [2] has a powerful oxidative action on the Fe II of myoglobin and oxymyoglobin [Mb(II)O_2]. As has been shown in [10], oxidation of Mb(II) by the action of H_2O_2 takes place 2 orders of magnitude faster than that of Mb(II)O_2. This reaction proceeds [4, 7, 8] by a two-electron mechanism with the formation of ferryl-myoglobin [Mb(IV)]. If the stoichiometric ratio between Mb(II)O_2 and H_2O_2 is 2:1 redox cascade is completed by the formation of metmyoglobin [(Mb(III)]:

$$\text{Mb (II) } O_2 \xrightarrow{K_1} \text{Mb (II)} + O_2 \tag{1}$$

$$\text{Mb (II)} + H_2O_2 \xrightarrow{K_2} \text{Mb (IV)} \tag{2}$$

$$\text{Mb (IV)} + \text{Mb (II)} \xrightarrow{K_3} 2\,\text{Mb (III)} \tag{3}$$

or taken together:

$$2\,\text{Mb (II)}\,O_2 + H_2O_2 \xrightarrow{K_4} 2\,\text{Mb (III)} + 2\,O_2 \tag{4}$$

The mechanism of electron transfer in the disproportionation reaction of Mb(II) and Mb(IV) is interesting, because on the basis of existing data [1, 5, 7, 8] it has been suggested that an electron conduction channel may exist in the apomyoglobin molecule, and that tyrosine (Tyr) residues, capable of participating in redox reactions with the formation of the radical form, are involved in its formation [5, 7]. It can accordingly be postulated that the derivatization of single (most accessible) Tyr residues in the myoglobin molecule leads to slowing of the velocity of reaction (3), and also to a decrease in the efficiency of the cascade process of oxidation of Mb(II)O_2 to Mb(III), described altogether by scheme (4), because modified Tyr residues are no longer able to participate in the oxidative free-radical process [4, 5], accompanying the step-by-step electron transfer between the heme group of Mb(II) and Mb(IV).

It is generally considered that quantitative derivatization of Tyr takes place on treatment of proteins with N-acetylimidazole [3, 6]. In the work cited, in which both myoglobin from sperm whale muscles (Tyr-103, Tyr-146, Tyr-151 [5]) and myoglobin from horse muscles (Tyr-103, Tyr-146 [5]) were used, showed that derivatization of 0.3-1.2 Tyr per protein molecule by N-acetylimidazole led to an increase in resistance of Mb(II)O_2 and Mb(II) to the oxidative action of H_2O_2.

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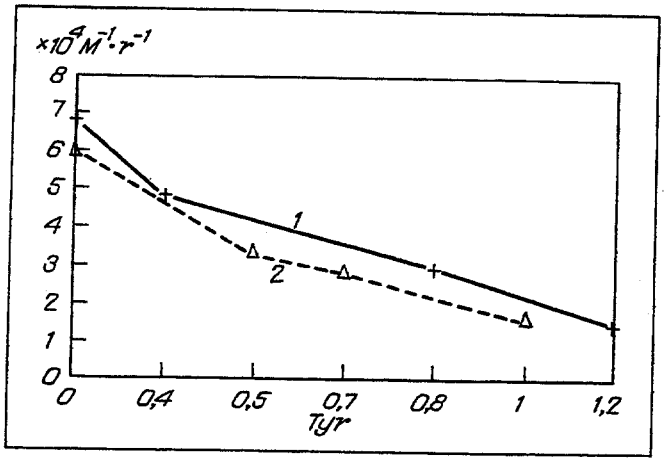

Fig. 1. Change in constant (K_4) of oxidation reaction of sperm whale (1) and equine (2) oxymyoglobin [Mb(II)O_2] under the influence of H_2O_2, depending on number of acetylated tyrosine residues of apoprotein (coefficients of correlation of 1 and 2 not less than 0.95).

EXPERIMENTAL METHOD

The investigation was carried out on myoglobin from the sperm whale (*Kogia breviceps*) and the horse (*Equus caballus*), produced by "Sigma" (USA). Myoglobin solutions were made up in 50 mM phosphate buffer, containing 1 mM EDTA, pH 7.4.

Deoxymyoglobin was obtained by reduction of a solution of commercial metmyoglobin by sodium dithionate ($Na_2S_2O_4$), followed by purification by gel-filtration on Sephadex G-75, using a column manufactured by "Pharmacia" (Sweden), as described in [8]. Deoxygenation of Mb(II)O_2 and oxidation of Mb(II) by the action of H_2O_2 was carried out in an atmosphere of nitrogen (high purity) in an anaerobic cuvette. The purity of the preparations was verified spectrophotometrically, by recording the absorption spectra of myoglobin in the region of 450-700 nm. The concentration of the hemoprotein in solution was verified with Drabkin's reagent ("Sigma," USA); a coefficient of molar absorption of the stained complex $\varepsilon_M = 11.3 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ at 540 nm was adopted in the calculations. The hydrogen peroxide concentration in the solutions was determined by measuring absorption at 240 nm ($\varepsilon_M = 43.6\ M^{-1} \cdot cm^{-1}$). Accumulation of Mb(III) in the reaction medium was monitored as the increase in absorption in the 502 nm region.

Ferryl-Mb(IV) was obtained as described in [8]. Derivatization of the tyrosine residues of myoglobin by N-acetylimidazole ("Sigma," USA) was carried out by the method described in [3, 6], with modifications. The myoglobin concentration in the incubation medium did not exceed 0.5 μM. Optimal concentrations of N-acetylimidazole were chosen in a preliminary series of experiments and were calculated so that the molar ratio of Mb(II)O_2 and N-acetylimidazole was 1:120 and 1:300 for sperm whale and equine myoglobin respectively. The modified myoglobin was purified by gel-filtration. The number of modified tyrosine residues was calculated on the basis of changes in absorption at 278 nm.

Spectral measurements were made on a Hewlett Packard 8452 A spectrophotometer with Hewlett Packard Vectra personal computer ("Multicell" HP 89531 A program).

EXPERIMENTAL RESULTS

The results of a preliminary investigation showed that derivatization of between 0.3 and 1.2 Tyr per myoglobin molecule did not lead to loss of the ability of Mb(II) to bind O_2, and likewise had no significant effect on resistance of heme iron(II) to undergo autooxidation in the process of oxygenation and deoxygenation. It follows from the data in Fig. 1 that derivatization of Tyr of myoglobin by N-acetylimidazole caused a decrease in K_4, i.e., reduced the reaction velocity of oxidation of Mb(II)O_2 to Mb(III) under the influence of H_2O_2. This effect for Mb(II)O_2 of both species of animals correlated with the number of modified Tyr groups in the myoglobin molecule. In fact, whereas K_4 of the reaction of oxidation of intact Mb(II)O_2 from sperm whale muscles was $(6.8 \pm 0.6) \cdot 10^4$, as a result of modification the values of K_4

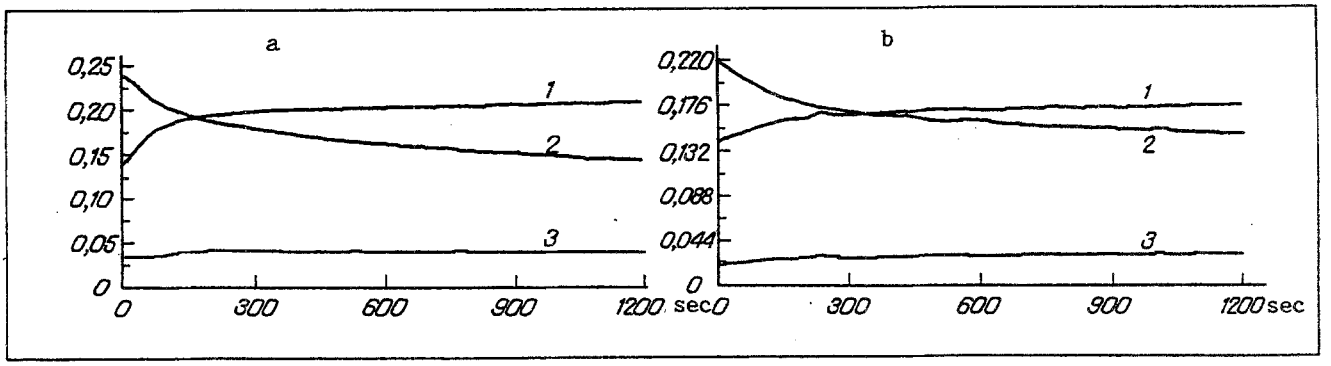

Fig. 2. Kinetics of change in content of intact sperm whale deoxymyoglobin (a) and of modified sperm whale deoxymyoglobin (b) in which 0.7 tyrosine residue was acetylated. Curve 1 represents deoxymyoglobin (552 nm), curve 2 – metmyoglobin (502 nm), 3) control (change in absorption of solution at 700 nm in the presence of H_2O_2).

fell to $(1.4 \pm 0.3) \cdot 10^4\ M^{-1} \cdot h^{-1}$ (acetylation of 1.2 Tyr). It follows from the graphic data shown in Fig. 1 that modification of about 1 Tyr both in $Mb(II)O_2$ from sperm whale muscles and in $Mb(II)O_2$ from equine muscles leads to an almost threefold decrease in K_4. The effect observed was evidently unconnected with a fall in K_2 (i.e., with a decrease in the velocity of oxidation of Mb(II) under the influence of H_2O_2), since a similar result was obtained for the use of Mb(IV) – the end product of reaction (2) – as oxidizer of $Mb(II)O_2$:

$$Mb\ (II)O_2 + Mb\ (IV) \xrightarrow{K_5} 2\ Mb\ (III) \qquad (5)$$

Calculation of the constant of reaction (5) showed that in the case of myoglobin from sperm whale muscles the presence of about 0.7 modified Tyr per molecule of $Mb(II)O_2$ led to a decrease in K_5 from $(7.4 \pm 0.3) \cdot 10^4$ to $(3.9 \pm 0.2) \cdot 10^{-4}\ M^{-1} \cdot h^{-1}$.

In turn, the kinetics of reaction

$$2\ Mb\ (II) + H_2O_2 \longrightarrow 2\ Mb\ (III) \qquad (6)$$

is evidence that acetylation of 0.8-1.0 Tyr residue per myoglobin molecule led to a change in the value of K_6 from $(4.3 \pm 0.5) \cdot 10^6$ to $(1.5 \pm 0.2) \cdot 10^6\ M^{-1} \cdot h^{-1}$ – when equine Mb(II) was used; and from $(3.7 \pm 0.4) \cdot 10^6$ to $(1.2 \pm 0.2) \cdot 10^6\ M^{-1} \cdot h^{-1}$ when sperm whale Mb(II) was used.

Comparison of the kinetics of reactions (6) and (4) indicates that a change in the velocity of reaction (6) is not the cause of the decrease in K_4. In fact, considering the high degree of the differences in the values of K_4 and K_6 (by almost 2 orders of magnitude) it can be concluded that the limiting stage of the cascade process (4) is deoxygenation of $Mb(II)O_2$. A change in K_6 could be reflected percepitibly in process (4) only if there were an approximately 100-fold decrease in K_6. In the experiment carried out acetylation of Tyr was accompanied by an only threefold decrease in K_6. Evidently modulation of K_6 as a result of acetylation of Tyr was due to a change in the effectiveness of electron exchange in the disproportionation reaction involving heme iron(II) of one myoglobin molecule and heme iron(IV) of the other. Incidentally, the observed effect of a decrease in K_6 as a result of acetylation of Tyr was identical in character both to that for myoglobin from sperm whale muscles and for myoglobin from equine muscles. Consequently, the degree of the decrease in K_6 did not depend on the total number of possible electron carriers, evidence in support of the existence of an intramolecular electron-transport chain.

The results thus confirm the involvement of tyrosine residues of the protein moiety of myoglobin in the reaction of disproportionation of heme iron(II) and (IV). This, in turn, suggest that preference can be awarded to a chemical mechanism of electron transport rather than the tunnel mechanism of electron transport [5, 7].

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SOME MECHANISMS OF INVOLVEMENT OF OPIOID PEPTIDES IN THE REGULATION OF CARBOHYDRATE METABOLISM

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Endogenous opioid peptides (OP) can influence many functions of the body. It has been reported that enkephalins and endorphins can prevent glycogenolysis and hyperglycemia, induced by adrenalin and parathyroid hormone [4, 5, 12]. OP also significantly alter secretion of catecholamines, cortisol, parathyroid hormone, and also several other hormones which influence carbohydrate metabolism [1, 7, 5], a fact which also suggests that they may be involved in the regulation of carbohydrate metabolism.

Nevertheless, the mechanisms of these effects of OP and, in particular, the role of different types of opiate receptors, in the realization of the action of enkephalins and endorphins on the parameters of carbohydrate metabolism still remain unexplained. For this reason research in this direction using selective agonists of mu- and delta-opiate receptors is of definite interest. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 141 male Wistar rats weighing 200-250 g. The OP for study and the adrenalin hydrochloride were injected intraperitoneally in doses of 0.5 mg/kg, dissolved in 0.5 ml of isotonic NaCl solution. Rats of the control group received an injection of the same volume of NaCl solution. The following reagents were used in the work: leucine-enkephalin (LE) was obtained from "Fluka" (Switzerland), the enkephalin analogs Tyr-D-Ala-Gly-Phe-D-Leu (DADLE), Tyr-D-Ala-Gly-(Me)Phe-Gly-ol (DAGO), and Tyr-D-Ala-Gly-Phe-Leu-Arg (dalargin) were obtained from the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR; Tyr-D-Arg-Gly-Phe-OEt (ee[D-Arg^2,des-Leu^5]enkephalin (the ethyl ester of [D-Arg^2,des-Leu^5]-enkephalin) was synthesized in the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR. The animals were killed by decapitation under superficial ether anesthesia 30 min after injection of the compounds. The glucose concentration in the blood plasma

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