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Original Contribution

Intra- and intermolecular oxidation of oxymyoglobin and oxyhemoglobin induced by hydroxyl and carbonate radicals

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Abstract

The mechanism of the reactions of myoglobin and hemoglobin with $^{\bullet}OH$ and $CO_3^{\bullet-}$ in the presence of oxygen was studied using pulse and γ-radiolysis. Unlike $^{\bullet}NO_2$, which adds to the porphyrin iron, $^{\bullet}OH$ and $CO_3^{\bullet-}$ form globin radicals. These secondary radicals oxidize the Fe^{II} center through both intra- and intermolecular processes. The intermolecular pathway was further demonstrated when BSA radicals derived from $^{\bullet}OH$ or $CO_3^{\bullet-}$ oxidized oxyhemoglobin and oxymyoglobin to their respective ferric states. The oxidation yields obtained by pulse radiolysis were lower compared to γ-radiolysis, where the contribution of radical-radical reactions is negligible. Full oxidation yields by $^{\bullet}OH$-derived globin radicals could be achieved only at relatively high concentrations of the heme protein mainly via an intermolecular pathway. It is suggested that $CO_3^{\bullet-}$ reaction with the protein yields Tyr and/or Trp-derived phenoxyl radicals, which solely oxidize the porphyrin iron under γ-radiolysis conditions. The $^{\bullet}OH$ particularly adds to aromatic residues, which can undergo elimination of H_2O forming the phenoxyl radical, and/or react rapidly with O_2 yielding peroxyl radicals. The peroxyl radical can oxidize a neighboring porphyrin iron and/or give rise to superoxide, which neither oxidize nor reduce the porphyrin iron. The potential physiological implications of this chemistry are that hemoglobin and myoglobin, being present at relatively high concentrations, can detoxify highly oxidizing radicals yielding the respective ferric states, which are not toxic.

Keywords: Globin radical; $^{\bullet}OH$ radical; Carbonate radical; Radiolysis; Kinetics; Heme protein; BSA; Hole tunneling

Introduction

The nature and fate of globin radicals have attracted a lot of interest particularly because they are formed alongside peroxide-mediated oxidation of heme proteins [1–3]. In these processes the formation of the relatively stable ferryl-oxo species is accompanied by generation of globin radical via Reactions (1)–(3) [4–8]:

$$\text{globin-Fe}^{III}\text{OH}_2 + H_2O_2 \rightarrow \text{globin-Fe}^{III}\text{OOH} + H^+ + H_2O \quad (1)$$

$$\text{globin-Fe}^{III}\text{OOH} \rightarrow \text{globin-Fe}^{V} = O + OH^- \quad (2)$$

$$\text{globin-Fe}^{V} = O \rightarrow {}^{\bullet}\text{globin-Fe}^{IV} = O \quad (3)$$

The identification of the sites of the amino acid radicals and the mechanism of their formation revealed an effective electron tunneling to the prosthetic heme group and a dynamic rearrangement of the protein radical among the amino acid residues [8–16]. This intramolecular process involves a sequential formation of radicals within the globin [8,9,11]. Certain residues were identified as the protein radicals, including Tyr [9,10,12,17], Trp [12,15,18], Phe [9], Cys [12], and His [19,20]. The fate of the globin radicals is yet poorly understood, and they decay via both intra- and intermolecular processes leading to modification and/or destruction of the heme protein [21,22] through disproportionation [17] or cross-linking [5,23,24]. In addition, these

Abbreviations: $ABTS^{2-}$, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate); BSA, bovine serum albumin; $HbFe^{III}OH_2$, methemoglobin; $MbFe^{III}OH_2$, metmyoglobin; $HbFe^{II}O_2$, oxyhemoglobin; $MbFe^{II}O_2$, oxymyoglobin; PB, phosphate buffer; Tyr, tyrosine; Trp, tryptophan; $Trp^{\bullet}$, tryptophan indolyl radical; Phe, phenylalanine; Cys, cysteine; His, histidine; Met, methionine.

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globin radicals can oxidize different substrates, such as styrene [6,25] and bovine serum albumin (BSA) [26]. Some, though not the phenoxyl radicals, combine with O_2 to form peroxyl radicals [6,9,10,17,18,27] that can act as oxidizing species [27].

Globin radicals were shown to be formed directly through the reaction of heme proteins with oxidizing radicals, such as $^{\bullet}OH$ [28–34] and $CO_3^{\bullet-}$ [35,36] and nonheme proteins with $^{\bullet}OH$ [28–34], $CO_3^{\bullet-}$ [35,36], and $^{\bullet}N_3$ [34,37]. Previous studies demonstrated that $^{\bullet}OH$ reaction with the globin of metmyoglobin [30] and metmyoglobin [31] under anoxia leads to reduction of the iron center. The reduction was ascribed to an intramolecular electron transfer from a globin radical to the heme iron [29–31], which does not occur in the presence of oxygen [31]. For the reduction process, a tunneling mechanism or an alternative process of fast consecutive intramolecular steps from remote radical sites to the iron center has been considered [29–31]. Analogously, the oxidation of oxyhemoglobin and oxymyoglobin to the corresponding ferric oxidation state by $CO_3^{\bullet-}$ has been suggested to occur through a rapid intramolecular process [35]. This process might reflect a "hole" tunneling process manifesting a long-range electron tunneling in the opposite direction, which resembles electron transfer throughout the globin that entails peroxide-induced oxidation of the heme protein [9,17]. Eventually, the formation of globin radicals can lead to oxidation/reduction of the iron through intra- and/or intermolecular processes. The present research concentrated on the mechanism of these processes initiated by the reaction of myoglobin and hemoglobin with $^{\bullet}OH$ and with the more selective $CO_3^{\bullet-}$ using steady-state and pulse radiolysis.

Materials and methods

Materials

All chemicals were of analytical grade and were used as received. Solutions were prepared with distilled water, which was further purified using a Milli-Q water purification system. Hemoglobin from human, myoglobin from horse heart, BSA, lysozyme from egg white and 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonate) ($ABTS^{2-}$) were obtained from Sigma. Catalase (2 mg/ml, about 130,000 U/ml) was obtained from Boehringer Mannheim. Isolated methemoglobin ($HbFe^{III}OH_2$) or metmyoglobin ($MbFe^{III}OH_2$) were prepared by adding an excess of ferricyanide to hemoglobin or myoglobin in 5–50 mM phosphate buffer (PB) at pH 7 followed by chromatographic separation through a Sephadex G-25 column. Oxyhemoglobin ($HbFe^{II}O_2$) or oxymyoglobin ($MbFe^{II}O_2$) solutions were similarly prepared by adding an excess of dithionite, kept in ice under nitrogen and used fresh on the same day. Solutions for γ-radiolysis experiments were saturated with N_2O and were mixed prior to irradiation with aerated solutions containing the protein and catalase so that under all experimental conditions the concentration of N_2O was higher than 14 mM while that of O_2 did not exceed 0.11 mM.

Spectrophotometric determinations

UV-visible absorption spectra of the heme proteins were monitored using 1, 2, or 10-mm optical path lengths and a Hewlett Packard 8453 diode array spectrophotometer. Concentrations of $MbFe^{II}O_2$ (ε_{543} = 13.9 mM^{-1} cm^{-1}), $MbFe^{III}OH_2$ (ε_{503} = 10.2 mM^{-1} cm^{-1}), $HbFe^{II}O_2$ (ε_{415} = 125 mM^{-1} cm^{-1} or ε_{541} = 13.8 mM^{-1} cm^{-1}, per heme), and $HbFe^{III}OH_2$ (ε_{405} = 179 mM^{-1} cm^{-1}, per heme) were determined from the respective absorption spectra monitored at pH 7.0 [38]. Unless otherwise stated, the concentrations and the rate constants for hemoglobin were expressed per heme.

Methods

Pulse radiolysis experiments were carried out using a 5-MeV Varian 7715 linear accelerator (0.1- to 0.5-μs electron pulses, 200 mA current). A 200 W Xe lamp produced the analyzing light. Appropriate cutoff filters were used to minimize photochemistry. All measurements were made at room temperature using either 1- or 2-cm spectrosil cells and applying three light passes. The dose per pulse was determined with the Fricke dosimeter using $G(Fe^{3+}) = 1.56 \times 10^{-6}$ M Gy^{-1} and $\varepsilon_{302}(Fe^{3+}) = 2197$ M^{-1} cm^{-1}. At least three experiments were averaged to obtain the observed rate constant.

Steady-state γ-irradiation experiments were carried out at room temperature using a ^{137}Cs source. The dose rate was determined by the Fricke dosimeter to be 9.4 Gy min^{-1}.

Results

Reaction with hydroxyl radicals

Hydroxyl radicals were generated upon irradiation of aqueous solutions containing 2.4 mM PB at pH 7.3 and saturated with $N_2O/O_2 = 9$, i.e., $[N_2O]$ = 22.5 mM, $[O_2]$ = 0.12 mM. Under such conditions e_{aq}^- and $H^{\cdot}$ formed by the radiation (Eq. (4)) are converted into $^{\bullet}OH$ (Reaction (5)) and $O_2^{\bullet-}$ (Reaction (6)), respectively. In Eq. (4) the values in parentheses are G values, defined as the number of species produced by 100 eV of energy absorbed (G = 1 equals 10^{-7} M Gy^{-1}).

$$H_2O \xrightarrow{\gamma} e_{aq}^- \ (2.6),\ {}^{\bullet}OH\ (2.7), H^{\bullet}\ (0.6),\ H_2\ (0.45), H_2O_2\ (0.7),\ H_3O^+\ (2.6) \quad (4)$$

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^{\bullet}OH + N_2 + OH^- \quad k_5 = 9.1 \times 10^9 M^{-1}s^{-1}\ [39] \quad (5)$$

$$H^{\bullet} + O_2 \rightarrow H^+ + O_2^{\bullet-} \quad k_6 = 1.2 \times 10^{10} M^{-1}s^{-1}\ [39] \quad (6)$$