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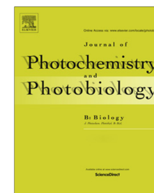

Heme degradation upon production of endogenous hydrogen peroxide via interaction of hemoglobin with sodium dodecyl sulfate

N. Salehi [a], A.A. Moosavi-Movahedi [a,b,*], L. Fotouhi [a], S. Yousefinejad [a], M. Shourian [a], R. Hosseinzadeh [a], N. Sheibani [c], M. Habibi-Rezaei [d]

[a] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[b] Center of Excellence in Biothermodynamics, University of Tehran, Tehran, Iran
[c] Departments of Ophthalmology and Visual Sciences and Pharmacology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[d] Schools of Biology, University of Tehran, Tehran, Iran

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ABSTRACT

In this study the hemoglobin heme degradation upon interaction with sodium dodecyl sulfate (SDS) was investigated using UV–vis and fluorescence spectroscopy, multivariate curve resolution analysis, and chemiluminescence method. Our results showed that heme degradation occurred during interaction of hemoglobin with SDS producing three fluorescent components. We showed that the hydrogen peroxide, produced during this interaction, caused heme degradation. In addition, the endogenous hydrogen peroxide was more effective in hemoglobin heme degradation compared to exogenously added hydrogen peroxide. The endogenous form of hydrogen peroxide altered oxyHb to aquamethemoglobin and hemichrome at low concentration. In contrast, the exogenous hydrogen peroxide lacked this ability under same conditions.

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1. Introduction

Hemoglobin (Hb), the major protein component of erythrocytes, is responsible for oxygen carrying from the lungs to respiring tissues [1]. Hemoglobin is a tetrameric allosteric protein that has a 3D structure consisting of two alpha and two beta subunits, which are non-covalently associated within erythrocytes and arranged around a central cavity [2]. Valuable reviews have been published on protein–surfactant interactions [3,4], and have evaluated surfactants' multi-step binding isotherms [5]. Other reviews by Jones [6], Randolph and Jones [7], and Moosavi-Movahedi [8,9] have focused especially on the thermodynamics of sodium dodecyl sulfate (SDS)–protein interactions. In addition, Otzen made efforts to overview different techniques studying protein surfactant interactions, and the behavior of different proteins in the contexts of surfactants [10].

Sodium dodecyl sulfate is an anionic surfactant used in many cleaning products and detergents, and also used in some foods and cosmetic products. This well-known surfactant has also been applied in pharmaceutical products as microbicide against various viruses including herpes simplex and human immunodeficiency virus [11,12], or as laxative and excipient [13,14]. The cleaning and cosmetic products come in contact with the surface of living organisms [8] and could straightly absorb into blood stream without filtering. Interaction of Hb with SDS at low concentration can result in increased metHb redox potential, which induces the sixth coordinated water oxidation and consequent metHb reduction [15]. In addition, the charge and pH values can impact the formation of hemichrome under the critical micelle concentration (CMC) of the surfactant. Furthermore, the interaction of SDS with Hb could be both electrostatic and hydrophobic, when pH is lower than the pI [16].

Each person has approximately 750 g of Hb, and 375 mg of heme content is degraded per day. Approximately 300 mg or 80% of degraded heme is produced from Hb [17]. In the body, there are two main pathways which result in heme degradation, enzymatic and non-enzymatic. Heme oxygenase is responsible for enzymatic degradation of the heme in most cells [18]. However, the adult red cells and serum have no heme oxygenase and heme is carried to the reticuloendothelial system of the liver, spleen and kidney for degradation [19].

In non-enzymatic heme degradation, oxy-hemoglobin (oxyHb) experiences redox reaction of heme iron with oxygen that produces reactive oxygen species (ROS). Heme proteins are a source

* Corresponding author at: Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. Tel.: +98 21 66403957; fax: +98 21 66404680.
E-mail address: moosavi@ut.ac.ir (A.A. Moosavi-Movahedi).

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of ROS that are thought to be involved in the deleterious effects under various disease states and during aging [17,20]. Non-enzymatic heme degradation has been already studied several decades ago. In 1937, Fisher and Muller reported degradation of heme by H_2O_2 for the first time [21]. The reaction of hydrogen peroxide with oxyHb (Fe (II)) and met-hemoglobin [metHb, Fe (III)] causes the formation of ferrylhemoglobin [ferrylHb; HbFe (IV)═O] and oxoferrylhemoglobin [oxoferrylHb; ·HbFe (IV)═O], respectively [22,23].

It was suggested by Nagababu and Rifkind [24] that the reaction between Hb and hemin with hydrogen peroxide coincides with production of two fluorescent compounds which have excitation wavelengths of 321 nm and 460 nm, and emission wavelengths of 465 nm and 525 nm, respectively. The fluorescence spectra of these products are clearly separate from the fluorescence of protoporphyrin without the iron that has a fluorescence excitation wavelength of 400 nm with an emission wavelength of 619 nm [17].

Heme degradation also occurs during autoxidation of oxyHb, producing superoxide and metHb [17,25]. Approximately 3% of oxyHb experience autoxidation each day producing superoxide (O_2^-), which could be converted to hydrogen peroxide by superoxide dismutase (SOD). The steady-state concentration of hydrogen peroxide in red blood cells is approximately 2×10^{-10} M [25]. FerrylHb and oxoferrylHb are formed by the reaction of Hb with hydrogen peroxide. They are strong oxidizing agents and unstable species. The ferrylHb could react with another molecule of hydrogen peroxide to produce superoxide, which is responsible for heme degradation. However, the reaction of metHb with hydrogen peroxide generates oxoferrylHb. In this reaction, oxygen produced instead of superoxide, and no heme degradation occurs [23].

Potassium superoxide in a protic solvent systems [23] and organic hydroperoxides could degrade heme and protoporphyrin to produce the same fluorescent products. The pH is also an effective factor for the production of fluorescent degradation products [17]. The reaction of linoleic hydroperoxide with hematin could generate ferryl heme [Fe (IV)-heme] or heme [Fe(II)-heme] [26]. The reaction of oxyHb with SDS leads to the formation of a superoxide radical. This suggests that SDS probably proceeds into the heme pocket and induces a conformational change [15,27].

In the present work, non-enzymatic degradation of heme during interactions of SDS with oxyHb was investigated. We found that the superoxide produced endogenously during this reaction generated heme degradation products.

2. Materials and methods

2.1. Materials

Sodium dodecyl sulfate (SDS, Sigma, 99%), Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Merck), potassium periodate (KIO_4, Merck), Sodium persulfate ($Na_2S_2O_8$, Merck), cupric sulfate ($CuSO_4{\cdot}5H_2O$, Merck), hydrogen peroxide (H_2O_2, 30% solution, Merck standardized by a UV–vis spectrophotometer at 240 nm), potassium hydroxide (KOH, Merck) and catalase (Merck, after centrifugation, standardized by a UV–vis spectrophotometer at 405 nm) were used. All solutions were prepared with double distilled water.

2.2. Hemoglobin preparation

Blood was collected from a healthy and non-smoker donor of genotype HbA according to the method of William and Tsay [28], and was stripped of anions as reported by Riggs [29]. Blood was centrifuged to remove plasma components. The packed red cells were washed by adding ten volumes of an isotonic saline solution (0.9% NaCl) and centrifuged at 4 °C for 15 min at 10,000 rpm. After removing the supernatant, five volumes of phosphate buffer (200 mM, pH 7.4) was added to the sample and centrifuged at 5000 rpm for 15 min. The washed packed cells were lysed with five volume of deionized water and centrifuged at 4 °C for 10 min at 18,000 rpm. In this step, stroma was discarded. The Hb solution was then brought to 20% saturation with ammonium sulfate, left standing for 15 min, and centrifuged at 2 °C for 1 h at 14,000 rpm. The recovered supernatant was then dialyzed by phosphate buffer (50 mM, pH 7.4) at 4 °C for 48 h, which was changed every seven hour. Concentration of Hb sample was determined from its absorbance at 541 and 415 nm using heme absorption coefficients (ε) of 13.8 and 125 $mM^{-1}\,cm^{-1}$ for aforementioned wavelengths, respectively. The SDS–PAGE (15%) and catalase test were used to confirm the purity of Hb (data not shown).

2.3. Fluorescence formation during the reaction of SDS with Hb

Fluorescence measurements were made using a fluorescence spectrophotometer (Cary Eclipse, Varian Co., Australia). Fluorescent emission spectra were scanned from 330 to 600 nm at excitation wavelength (Ex) of 321 nm and from 470 to 700 nm at excitation wavelength of 460 nm, at 25 °C and 37 °C. The time dependent generation of emission (Em) spectra was scanned with 5 min intervals in 1 h from 330 nm to 600 nm with Ex wavelength of 321 nm as well as from 470 nm to 700 nm with Ex wavelength of 460 nm. In all fluorescence experiments, the width of both Ex and Em slits of the instrument were set at 10 nm.

2.4. Curve resolution analysis

To obtain more information about the heme degradation process, a chemometric analysis was performed on the fluorescence data of Hb in the presence of different amounts of SDS. Multivariate curve resolution alternating least squares (MCR-ALS) as a well-known curve resolution method [30,32] was used to extract information from the fluorescence data. Alternatively, augmentation strategy could be a powerful method in both aspects of increasing information and decreasing ambiguity in MCR-ALS [33]. In such a strategy, multiple independent experiments under different conditions are analyzed simultaneously. If m samples excite in j excitation wavelength and their emission record in a range of n wavelength, then we could collect data in j matrices of the size of $m \times n$.

Here two sets of emission spectra of the Hb (50 μM) incubated with 31 different concentrations of SDS (ranging from 0 to 2 mM) were collected. First, the emission in the range of 400–546 nm (with increment = 2 nm) excited in 6 different wavelengths (305, 310, 315, 320, 325 and 330 nm). These six excitation wavelengths were selected around the well-known 321 nm [24] to detect heme degradation product(s), which appear in the emission range of 400–546 nm. Thus, the first data set contained 6 data matrices with the size of 31×74. The second data set contained 7 data matrices which resulted in excitation of 31 samples in 7 different wavelengths (450, 455, 460, 465, 470, 475 and 480 nm) and collecting the emission in the range of 516–680 nm (with increment = 2 nm). These seven excitation wavelengths were chosen around 460 nm [24] to recognize heme degradation product(s), which appear in the emission range of 516–680 nm. Thus, each of these seven data matrices were of the size of 31×83. These augmented data matrices were then subjected to factor analysis to evaluate the number of components. For this purpose, singular-value decomposition (SVD) was used [34].

The details of MCR-ALS could be found elsewhere [30]. Briefly, it should be noted that in this method the original data matrix is decomposed to two absolute scores, and loading matrices in the

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