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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

^aInstitute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

^bCenter of Excellence in Bio thermodynamics, University of Tehran, Tehran, Iran

^cDepartments of Ophthalmology and Visual Sciences and Pharmacology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

^dSchools of Biology, University of Tehran, Tehran, Iran

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 hemi-chrome at low concentration. In contrast, the exogenous hydrogen peroxide lacked this ability under same conditions.

Keywords

Hemoglobin; Sodium dodecyl sulfate; Heme degradation; Endogenous hydrogen peroxide; Chemiluminescence; Multivariate curve resolution

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

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

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jphotobiol.2014.02.014>.

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 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 (KIO₄, Merck), Sodium persulfate (Na₂S₂O₈, Merck), cupric sulfate (CuSO₄ 5H₂O, Merck), hydrogen peroxide (H₂O₂, 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 14,000rpm. 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 μ M) 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 direction of rows and columns of the original data, respectively. However some chemical/physical constraints were used in an iterative process to give meaningful results. On the other words, using suitable constraints helped to collect concentration and spectral information in the score and loading matrices, respectively. The decomposition of a

data matrix like $\mathbf{D}$ could be abstracted in $\mathbf{D} = \mathbf{CS}^T + \mathbf{E}$. Variation of each contribution in the row and the column directions of $\mathbf{D}$, by matrices $\mathbf{C}$ and $\mathbf{S}^T$, respectively. Thus, $\mathbf{C}$ is the concentration profile, $\mathbf{S}$ is the spectral profile, and $\mathbf{E}$ is the residual matrix with the same size of $\mathbf{D}$. The 'T' superscript on $\mathbf{S}$ denotes the transpose of spectral profile ($\mathbf{S}$).

An initial estimate is required in this iterative method. We could use an estimate of concentration ($\mathbf{C}$) or pure spectra ($\mathbf{S}$) as the initial estimate. Evolving factor analysis (EFA) and simple-to-use interactive self-modeling mixture analysis (SIMPLISMA) are two well-known methods for calculation of the initial estimates of $\mathbf{C}$ and $\mathbf{S}$, respectively [35,36]. Here the results of MCR-ALS using EFA are presented. However, similar results were obtained using SIMPLISMA. During the alternative least square, non-negativity and unimodality constraints were applied on the concentration profile, and non-negativity was carried out on the spectral profile. The details and benefits of these constraints could be found in the literature [37]. All data manipulations were performed in MATLAB environment (version 7, Math work, Inc., <http://www.math-works.com>, USA) and the codes written by Tauler research group [31] were used for curve resolution analysis.

2.5. Chemiluminescence spectroscopy

In order to perform chemiluminescence experiments, luminol solution, Hb and reactant (SDS or hydrogen peroxide) were dissolved in sodium carbonate buffer (100 mM, pH 11). Chemiluminescence signal was recorded at 425 nm after injecting 5 μ l of diperiodatocuprate (III) (DPC) to the sample by a fluorescence spectrophotometer (Synergy H4 Hybrid Reader; BioTek, USA). To prepare DPC stock solution (10 mM) based on the protocol reported by Hu et al. [38], a proper amount of KIO_4 (0.023 g), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.0125 g), $\text{Na}_2\text{S}_2\text{O}_8$ (0.014 g) and KOH (0.08 g) were added to 3 ml of water. The mixture was boiled for 20 min and then chilled to room temperature. Finally, the mixture was diluted to 5 ml and was stored at 4 °C [38,39]. The stock solution of luminol (10 mM) was prepared in sodium carbonate buffer (100 mM, pH 11), stored at 4 °C for 48 h in dark condition, and diluted to 10^{-5} M for further use in chemiluminescence spectroscopy.

2.6. UV-vis spectrophotometry

The UV-vis spectra of Hb (5 μ M) with hydrogen peroxide (0–2 μ M) in phosphate buffer solution were recorded using a UV-vis spectrophotometer (Cary 100, Varian Co., Australia) at 25 °C in the range of 450–700 nm.

3. Results and discussion

3.1. Detection of heme degradation products

In the current study, a fluorescence technique was used to detect the heme degradation products during SDS reaction with Hb according to the work of Nagababu and Rifkind [24]. We found that during Hb reaction with different concentrations of SDS in phosphate buffer (50 mM, pH 7.4) at 25 °C some fluorescent degradation products were formed. When the solution was excited at 321 and 460 nm Hb displayed fluorescence emission peaks around 465 and 525 nm, respectively (Fig. 1), and the addition of SDS led to a distinct increase in the fluorescence signal (SDS do not have any fluorescence peak in this area).

However, the fluorescence at 550 nm shown in Fig. 1b is the Raman band originating from the buffer [24]. Therefore, the existence of these fluorescence bands are a clear indication that the heme is being degraded in the presence of SDS.

The fluorescent bands were the same as the fluorescent bands achieved during the reaction of oxyHb with hydrogen peroxide [23], and during auto-oxidation of oxyHb [38]. These results showed that these products are identical. According to the similarity of the fluorescence peaks obtained in our study and those resulted in the reaction of oxyHb or protoporphyrin IX with hydrogen peroxide [23] and autoxidation process of oxyHb [25], it could be concluded that some similar heme degradation products were formed due to SDS effects.

Fig. 2 shows the effect of time on the SDS and autoxidation of Hb, which induced Hb (50 μ M) heme degradation in the presence of a fixed amounts of SDS (0.5 mM). The samples were excited at both 321 and 460 nm, and their maximum fluorescence was recorded, every 1 h for a period of 8 h at 25 °C. This figure reveals that SDS increases the rate of autoxidation of Hb. The difference between SDS induced oxidation and autoxidation is more obvious in 321 nm product. The slope of heme degradation decreases after about 4 h of incubation. A fraction of Hb changed to metHb during interaction with SDS up to this time [16,40].

3.2. Curve resolution analysis

The main goal of this part of the study was to explore the real number of heme degradation products after SDS addition. The first data set from the excitation range of 305–320 nm and emission range of 400–546 nm were augmented in the row direction and are shown in Fig. 3a. The augmented data matrix was of the size of 31×444 . The row-wised augmentation was carried out since all the data matrices had the same concentration profile, and their emission profiles were different since different excitation wave-lengths were used. The MCR-ALS results are in a spectral profile (combination of 6 parts) and a single concentration profile with N component (data not shown). The SVD showed the presentation of 2 significant components in the augmented data set 1. After using appropriate constraint and doing ALS optimization, the spectra and concentration profile of the heme degradation products were resolved and is presented in Fig. 4. It is clear that both components were produced by adding SDS, and there are the products of heme degradation process. As it could be seen in the original fluorescence data (Fig. 3a) the intensity of Hb in the excitation range of 305–330 nm is low compared with the products of heme degradation. Fig. 4 indicates that the two heme degradation products were not generated simultaneously from the start of the reaction. One product was produced as the result of increased SDS concentration and extended with two different slopes. The other product just generated when SDS concentration reached 450 μ M.

To produce another data set, the Hb samples titrated with different amount of SDS were excited at 7 different excitation wave-lengths (ranging from 450 to 480 nm) and their emissions were recorded in the range of 516–680 nm. The 7 data matrices of the size of 31×83 were then row-wise augmented and were analyzed by SVD. The results showed that only one component causes variation in this data set. It should be noted that in addition to

decreasing the analysis ambiguity, another benefit of using different excitation wavelength is increasing the system information to resolve the compounds with high overlapped peaks. However, the results showed the existence of just one compound in this region and there was no need for curve resolution in this case. The augmented data (size = 31×581) are graphically represented in Fig. 3b. Thus, it was concluded that 3 fluorescent compounds were produced according to the SDS-induced heme degradation. Two of these were detected in the emission range of 400–546 nm and the other in the emission range of 450–480 nm. In the current research the simple fluorescence spectroscopy method combined with chemometrics was utilized to detect the three heme degradation products. These results were consistent with those recently reported by Maitra et al. [41], who showed the interaction of Hb with hypochloric acid produces three degradation species by mass spectrometry analysis.

3.3. Detection of hydrogen peroxide

The detection of the three fluorescent products during the Hb reaction with SDS suggested that the heme degradation may involve the reaction of ROS with oxyHb. To address this point a chemiluminescence study was performed. Based on the chemiluminescence theory of luminol, increasing the oxidizing agents, like hydrogen peroxide or ROS, leads to the raise of 3-aminophthlate concentration as an electronically excited state molecule. The chemiluminescence relays on the emission spectrum of the first singlet excited state of 3-aminophthlate, and the hydrogen peroxide appears to be the generator of this excited state intermediate molecule. When 3-aminophthlate undergoes a transition to the ground state emits a photon light in the wavelength of 425 nm.

Fig. 5a shows the chemiluminescence emission of luminol in purified Hb with different amount of SDS samples in absence and with added 25 nM catalase, which was proportional to the chemiluminescence intensity at 425 nm. Our results showed that chemiluminescence emission was enhanced with increasing of SDS concentration up to 1 mM, when the chemiluminescence intensity reached near constant level. Again this observation is due to the conversion of Hb to metHb in the presence of high SDS concentration [16,40]. Under such conditions, oxygen is produced instead of superoxide as the consequence of the reaction of metHb with exogenous hydrogen peroxide [23]. To understand if hydrogen peroxide was responsible for chemiluminescence emission enhancement, catalase (25 nM) was applied. As it is shown in Fig. 5a catalase caused decrease in the chemiluminescence emission about 90% in each point. This result means chemiluminescence emission was due to presence of hydrogen peroxide.

To have a quantitative insight into the amounts of hydrogen peroxide produced as a result of Hb interaction with SDS, a chemiluminescence calibration curve was obtained for hydrogen peroxide (inset in Fig. 5a). After injection of DPC to luminol- hydrogen peroxide solution the chemiluminescence intensity was dramatically increased. As shown in the inset of Fig. 5a there is a good linear relationship between the concentration of hydrogen peroxide and subsequent recorded chemiluminescence intensities, with a correlation coefficient of 0.997. The linear equation of this calibration (see inset in Fig. 5a) and chemiluminescence intensity obtained in the interaction of SDS and Hb (Fig. 5a) was used to estimate the amount of hydrogen peroxide produced in heme degradation process. The calculated concentration of

hydrogen peroxide formed by different amounts of SDS is represented in Fig. 5b. The hydrogen peroxide concentration was increased with increasing SDS concentration up to 1 mM, which produced approximately 2 μ M of hydrogen peroxide. After that, as it was expected from chemiluminescence intensities in Fig. 5a, the quantity of hydrogen peroxide did not dramatically change. It should be emphasized that the final hydrogen peroxide level found in this process (2 μ M) is higher than its steady-state amount in normal blood cells (2×10^{-4} μ M) [25].

3.4. Comparison of the effects of endogenous and exogenous hydrogen peroxide

To compare the induced effects of endogenous and exogenous hydrogen peroxide on the heme degradation, fluorescence intensity of the degradation products was measured by adding hydrogen peroxide exogenously at the same concentration that was produced endogenously in the presence of SDS. A comparison between the effect of endogenous and exogenous hydrogen peroxide on the production of heme degradation fluorescent compounds is illustrated in Fig. 6a. Based on these results, exogenous hydrogen peroxide (in low concentration of 2 μ M) induces negligible heme degradation. These results showed the greater effects of endogenous hydrogen peroxide on heme degradation of Hb.

The chemiluminescence was utilized to determine the effect of endogenous and exogenous hydrogen peroxide on heme degradation. Fig. 6b shows the obtained chemiluminescence intensity against endogenous (by adding SDS to Hb which induced hydrogen peroxide internally 0.2–2 μ M) and exogenous hydrogen peroxide (by adding hydrogen peroxide exogenously) at the same concentrations. The test was also repeated reverse mode, in which the chemiluminescence intensity was constant and the corresponding concentration of endogenous and exogenous hydrogen peroxide was examined (figure is shown in supplementary data). These results provide additional evidence for the greater effect of endogenous hydrogen peroxide produced during interaction of SDS with Hb compared with exogenous hydrogen peroxide. This considerable effectiveness might be because of the suitable vicinity of endogenous hydrogen peroxide to the reaction location.

To gain detailed insight into the differences between the effect of endogenous and exogenous hydrogen peroxide, we studied the effect of different amounts of exogenous and endogenous hydrogen peroxide on Hb by UV-vis spectrophotometry (Fig. 7a and b). The added hydrogen peroxides were equivalent to the amounts produced endogenously during the reaction of Hb with SDS. Previous studies showed changing of oxyHb to metHb, aquametHb and hemichrome due to the interaction of Hb with SDS [16,40], which are confirmed by Fig. 7a. Changes were observed in the ligand-LMCT band (approximately at 640 nm) or the Q band (around 500 nm) indicating generation of aquametHb species in the presence of endogenously produced hydrogen peroxide. In addition, appearing of a band at 535 nm and a shoulder at 565–575 nm was observed during the addition of endogenous hydrogen peroxide, which indicated production of hemichrome and the peak was observed around 580 which is indicator of ferrylHb [16,40,42]. The spectral decreasing in Fig. 7b indicates that exogenous hydrogen peroxide caused the changing of oxyHb to metHb. Thus, the interaction of endogenous and exogenous hydrogen peroxide with Hb had different results.

4. Conclusion

Based on our experimental results, it was confirmed that the interaction between Hb and SDS leads to heme degradation. Generation of hydrogen peroxide during this process was responsible for heme degradation in Hb which was confirmed by adding catalase (catalyzed H_2O_2) during chemiluminescence experiment. Comparison between the effect of SDS on Hb oxidation and autoxidation of Hb reveals that SDS increases the rate of autoxidation of Hb. It was shown by chemometrics via fluorescence spectra; three compounds were produced during this process that was in agreement with the literature in which three heme degradation products were determined experimentally [41]. The hydrogen peroxide produced endogenously during the interaction of Hb and SDS was more effective compared to exogenously added hydrogen peroxide to Hb. This was attributed to the changing of Hb to aquamethHb and hemichrome as a result of interaction with endogenous hydrogen peroxide.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Formation of fluorescent bands during the reaction of oxy-Hb (50 μM) with different concentrations of SDS 0 (A), 50 (B), 100 (C), 300 (D), 500 (E), 700 (F), 1000 (G), 1500 (H), 2000 (I) μM immediately in potassium phosphate buffer (50 mM, pH 7.4), at 25 $^{\circ}\text{C}$ after excitation at (a) 321 nm and (b) 460 nm.

Fig. 2. Effect of SDS (0.5 mM) and autoxidation on fluorescence intensity of heme degradation products of the reaction of hemoglobin (50 μ M) in phosphate buffer (50mM, pH 7), at 25 °C: (a) excitation wavelength of 321 (■), 460 (●) nm for adding SDS, and (b) excitation wavelength of 321 (□), 460 (○) nm for autoxidation at 25 °C with a time interval of 1h in 8h.

Fig. 3. Three dimensional representation of fluorescence intensity in different SDS additions related to the row-wised augmented data. (a) The emission range of each part is 400–546 nm; and (b) the emission range of each part is 516–680 nm. The excitation wavelength of each part is shown on the figure.

Fig. 4.

(a) The spectral profile of the row-wised augmented fluorescence data of heme degradation system. Each part of the profile shows the resolved spectra of two heme degradation products (C1 and C2) in the emission range of 400–546 nm. Excitation wavelength used for each part is noted in the figure. (b) Concentration profile of products vs. different concentrations of SDS.

Fig. 5.

Hydrogen peroxide producing during the reaction of hemoglobin (5 μM) with different concentrations of SDS (a) Observed chemiluminescence intensity in absence (♦) and with added 25 nM catalase (▲), (inset in a) calibration curve of hydrogen peroxide and (b) hydrogen peroxide generated during the reaction of hemoglobin with SDS.

Fig. 6.

Effect of endogenous and exogenous hydrogen peroxide (200, 450, 670, 1000, 1400, 1900, 2000, 2100 nM) on hemoglobin (50 μM) on: (a) formation of fluorescent products bands (endogenously at excitation wavelength of 321 (■), 460 (●) nm and exogenously at 321 (□), 460 (○) nm); and (b) chemiluminescence intensity (endogenous (▲) and exogenous (△)).

Fig. 7. UV-vis spectra of Hb (5.0 μM) with (a) SDS (0, 50, 100, 300, 500, 700, 1000, 1500, 2000 μM), and (b) hydrogen peroxide (0, 0.2, 0.45, 0.67, 1, 1.4, 1.9, 2, 2.1 μM) in potassium phosphate buffer, pH 7.4 (50 mM) at 25 °C.