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Interaction of sodium dodecyl sulfate with human native and cross-linked hemoglobins: a transient kinetic study

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Abstract

The interaction of sodium dodecyl sulfate (SDS) at a concentration range (0–515 μM) below the critical micelle concentration (CMC ~ 0.83 mM) with human native and cross-linked oxyhemoglobin (oxyHb) and methemoglobin (metHb) has been investigated by optical spectroscopy and stopped-flow transient kinetic measurements. It is observed that the interaction of SDS with human native and cross-linked oxyHb shows the disappearance of the bands of oxyHb at 541 and 576 nm and the appearance at 537 nm. The resultant spectra are characteristic of low spin (Fe^{3+}) hemichrome. Similarly SDS has been found to convert human native and cross-linked high spin (Fe^{3+}) metHb to low spin (Fe^{3+}) hemichrome. The interaction of SDS with oxyHb suggests a conformational change of the protein in the heme pocket, which may induce the binding of distal histidine to iron leading to the formation of superoxide radical. The formation of hemichrome from metHb is found to be concentration-dependent with SDS. The stopped flow transient kinetic measurements of the interaction of SDS with metHb show that at least four molecules of SDS interact with one molecule of metHb. The interaction of SDS with human cross-linked oxy and met hemoglobin shows results similar to those for human native oxy and met hemoglobin indicating that the covalent modification does not alter the interaction of SDS with cross-linked hemoglobin.

Keywords: Sodium dodecyl sulfate; Human native; Cross-linked hemoglobins

1. Introduction

Hemoglobin appears to be the main component of erythrocytes and ensures oxygen transport to all organs and parts of the body. It consists of two alpha and beta subunits. It is known to exist in at least two different molecular conformations, which correspond to the absence and presence of oxygen as the sixth ligand of the heme iron. The functionally active form contains a ferrous ion in a porphyrine ring. Oxygen binding to hemoglobin takes place at the ferrous heme center of each of the four monomeric units giving oxyhemoglobin (oxyHb) in the R-state of the protein. Release of oxygen from oxyHb leads to deoxyhemoglobin (deoxy Hb) which is characteristic of the T-state.

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It has been found that bacterial endotoxin (LPS) binds to oxyHb and enhances the pathogenic activity of LPS [1]. It is postulated that the interaction of LPS and free fatty acids with human and cross-linked hemoglobin leads to the formation of high spin (Fe^{3+}) methemoglobin and low spin (Fe^{3+}) hemichrome [1,2]. The long hydrophobic chain of fatty acids in LPS and free fatty acids might be responsible for this conversion [1]. It is observed that hemichrome accumulation in red cells is typical of some blood diseases [3,4] and aging of erythrocyte [5]. Since sodium dodecyl sulfate (SDS) has a long hydrophobic chain, it is expected that its effect on oxyhemoglobin may be similar to LPS and free fatty acids.

In order to understand whether SDS can induce protein structural changes in hemoglobin leading to its oxidation, we have studied the interaction of SDS with both native and chemically modified human hemoglobin by optical spectroscopy and stopped flow transient kinetic measurements. The results show that the interaction leads to the formation of hemichrome. Stopped flow kinetics of the formation of hemichrome from metHb suggest that four molecules of SDS interact with one molecule of methemoglobin.

2. Materials and methods

Human hemoglobin was prepared and purified, as described by Roosi Fanelli et al. [6]. Cross-linked hemoglobin was prepared from human hemoglobin by covalently cross-linking between Lys 99 residues of the alpha subunits with a bis(3,5-dibromosalicyl) group. Methemoglobin was prepared by oxidation of oxyhemoglobin with potassium ferricyanide. The reaction was carried out in the dark at 4 °C for approximately 30 min with occasional mixing. The molar ratio of potassium ferricyanide to oxyhemoglobin was 1.5:1. MetHb was separated from ferrocyanide and residual ferricyanide by sephadex G-25 column chromatography. The metHb stock solution was stored at 4 °C in 0.1 M sodium phosphate buffer (pH 7.0). SDS was obtained from sigma. The experiments with SDS were done in 0.1 M sodium phosphate buffer (pH 7.0).

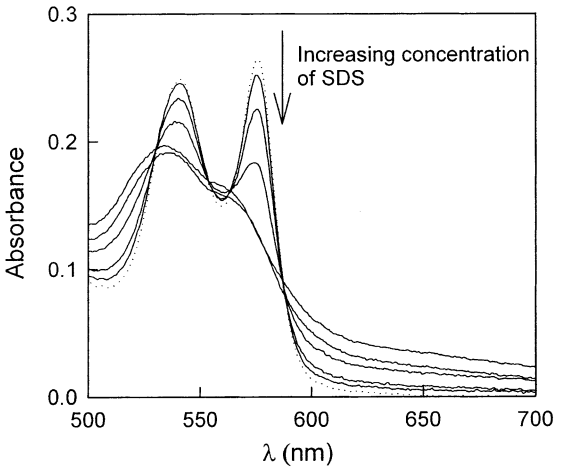

Fig. 1. Optical spectra of human hemoglobin (17.6 μM) at different concentrations of SDS. Dotted line is the optical spectrum of oxyhemoglobin. The concentration of SDS (100–500 μM) increases from top to bottom.

Spectrophotometric experiments were performed on a Shimadzu UV-2100 spectrophotometer. The stopped flow kinetic experiments were performed on a microprocessor controlled HITECH SF-61 stop flow machine. The transient kinetic studies of the formation of hemichrome were performed by loading the methemoglobin and SDS solutions in two separate syringes. The measurements were done spectrophotometrically by monitoring the wavelength of interest. The kinetic data for the rapid kinetics were analyzed by fitting to the exponential function using HITECH-developed software.

3. Results and discussion

3.1. Optical spectra

Fig. 1 shows the optical spectra of human native oxyHb with increasing concentration of SDS. The band at 576 nm is characteristic of oxyHb. On addition of SDS to oxyHb, this band disappears and a new band appears at 537 nm. The band at 537 nm is considered to be characteristic of hemichrome [1,2]. Kaca et al. [1] and Akhrem et al. [2] have studied the formation of hemichrome from the interaction of oxyhemoglobin with LPS

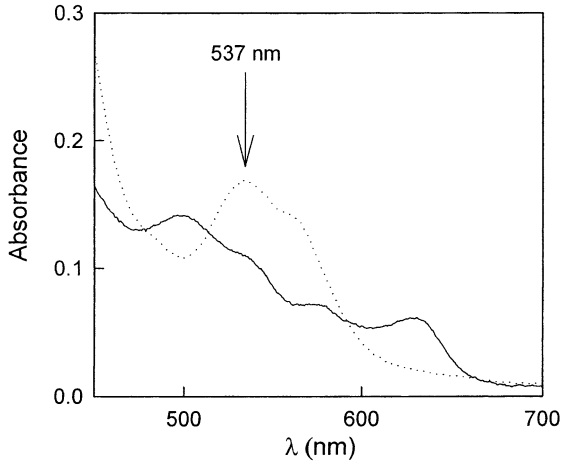

Fig. 2. Optical spectra of human methemoglobin (solid line) and human methemoglobin plus SDS (dotted line) in 100 mM sodium phosphate buffer (pH 7.0). The concentration of human methemoglobin and SDS were 17.6 and 492 μM, respectively.

and free fatty acids, and have reported the appearance of the band at 537 nm. Hemichrome is the oxidized product of hemoglobin with two histdines (proximal and distal) ligated to the six coordinated low spin heme irons. The conversion of oxyHb to hemichrome is found to be dependent on SDS concentrations. The interaction of SDS with high spin Fe^{3+} human metHb shows results similar to those for oxyhemoglobin (Fig. 2). We have also studied the interaction of SDS with human cross-linked oxyhemoglobin and metHb, and observed the formation of hemichrome as in the case of human native oxy and met hemoglobin.

3.2. Transient kinetics

Since the interaction of SDS with human native and cross-linked metHb showed an increase in the absorbance at 537 nm, the kinetics of the formation of hemichrome was investigated by the stopped flow technique monitoring the change in the absorbance at this wavelength. The kinetics of the formation of hemichrome from oxyHb could not be studied in detail due to the complex nature of the reaction. It was observed that the reaction did not complete over a period of several hours. This could be due to the auto-oxidation of hemoglobin. We have therefore studied the transient kinetics of the formation of hemichrome from the interaction of SDS with metHb. A typical stopped flow trace for the formation of hemichrome from human metHb is shown in Fig. 3. The data could be best fitted to three exponential functions, and pseudo-first order rate constants were obtained. The presence of the three exponential components in the transient kinetics implies three different types of interaction. These could be the breakage of the iron oxygen bond of water molecule coordinated to the heme, binding of the distal histidine to the heme and breakage of the hydrogen-bonding network of the distal histidine with the surrounding solvent.

A typical set of the data of the rate constants were found to be $k_{1,obs}=60.0\ s^{-1}$, $k_{2,obs}=19.5\ s^{-1}$ and $k_{3,obs}=2.8\ s^{-1}$ in 100 mM phosphate buffer, pH 7.0 when 372 μM SDS reacted with 8

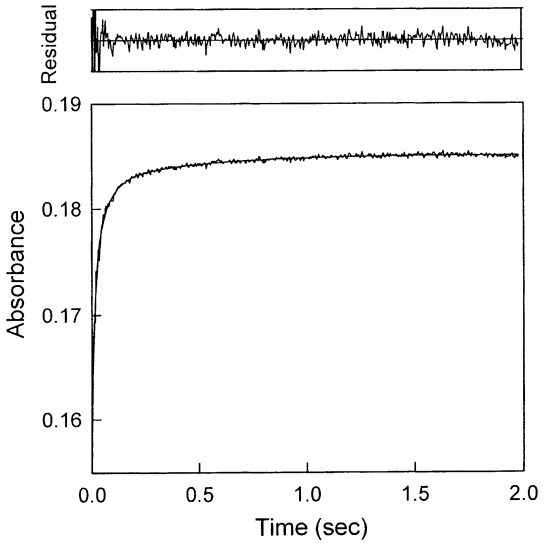

Fig. 3. Typical stopped flow trace for the increase in the absorbance at 537 nm due to the formation of hemichrome from human methemoglobin (8 μM) at 372 μM SDS at pH 7.0. The solid line drawn through the experimental trace is the computer fit to $A_t=A_1\exp(k_{1,obs}t)+A_2\exp(k_{2,obs}t)+A_3\exp(k_{3,obs}t)+B$. A_t is the absorbance at time t, $k_{1,obs}$, $k_{2,obs}$, and $k_{3,obs}$ are the pseudo-first order rate constants, and A_1, A_2 and A_3 are the corresponding amplitudes, and B is the equilibrium signal (offset). Upper curve in the figure is the residual ($A_{obs}-A_{calc}$) plot, which shows the accuracy of the computer generated fit.

μM metHb. $k_{1,obs}$ is found to be almost 20 and three times greater than $k_{3,obs}$ and $k_{2,obs}$, respectively. This indicates that the process corresponding to $k_{1,obs}$ takes place at a much faster time scale than the other two rate constants. We have no evidence that the rate constants may correspond to which interactions. However, it is possible that the rate constants for the breakage of the iron–oxygen bond of the water molecule coordinated to the heme and breakage of the hydrogen bonding network of the distal histidine with the surrounding solvent may be slower than the binding of distal histidine to the heme (ligand displacement reaction). Hence, k_1 may correspond to the binding of distal histidine to metHb, and k_2 and k_3 may correspond to other interactions.

We investigated the formation of hemichrome in the 150–470-μM concentration range of SDS. The kinetic traces fit well to three exponential functions yielding pseudo-first order rate constants at different concentrations of SDS. The dependence of these three rate constants on the SDS concentrations is shown in Fig. 4. The plot of $k_{1,obs}$ with increasing concentration of SDS shows non-linear and sigmoidal behavior. This type of behavior suggests that in the process corresponding to $k_{1,obs}$ more than one molecule of SDS is reversibly interacting with one molecule of metHb leading to the formation of hemichrome. A possible reaction scheme for the formation of hemichrome from metHb may be shown as

$$\text{MetHb} + n\text{SDS} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{MetHb}\cdot n\text{SDS} \xrightarrow{k_2} \text{hemichrome}$$

where $K_d = k_{-1}/k_1$ refers to the dissociation constant of MetHb·nSDS complex, k_2 is the rate constant for the formation of hemichrome and n is the number of SDS molecules. The value of n is greater than one. The following relation of $k_{1,obs}$ with SDS concentration can be derived from the above reaction scheme:

$$k_{1,obs} = \frac{k_2[\text{SDS}]^n}{K_d + [\text{SDS}]^n} \quad (1)$$

Assuming that the binding affinity of four SDS molecules to metHb is the same, the experimental data of Fig. 4a was fitted best to Eq. (1) with $n=4$. This suggests that one of the interactions involves reversible binding of four molecules of SDS to one molecule of metHb.

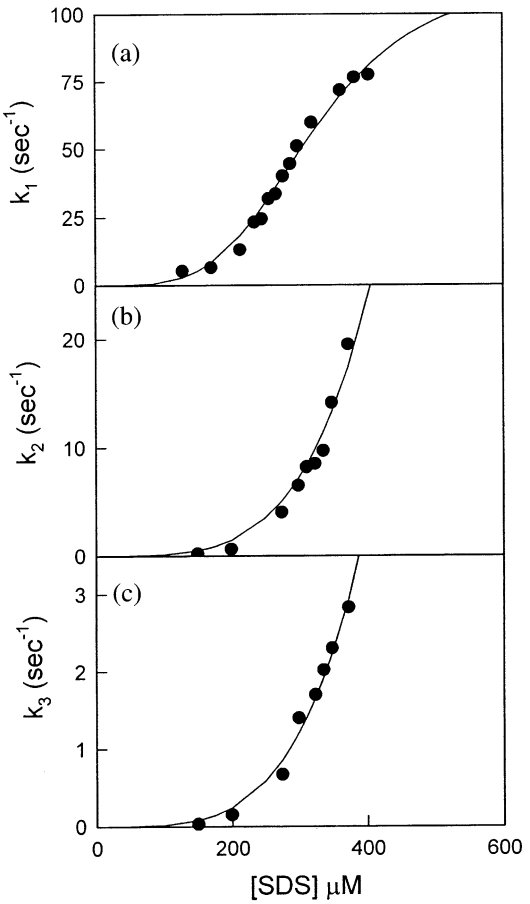

Fig. 4. (a) Plot of $k_{1,obs}$ vs. [SDS] at pH 7.0. The solid line drawn through the experimental data is the computer fit to Eq. (1). (b) Plot of $k_{2,obs}$ vs. [SDS]. (c) Plot of $k_{3,obs}$ vs. [SDS]. The solid lines drawn through the experimental data of (b,c) show the computer fit to Eq. (2).

$k_{2,obs}$ and $k_{3,obs}$ increase non-linearly with SDS concentration and no sigmoidal behavior is observed (Fig. 4b,c). The experimental data were fitted to the following equation:

$$k_{i,obs} = k[\text{SDS}]^n \qquad i = 2,3 \quad (2)$$

where k is the rate constant for the formation of hemichrome from metHb. The data gave a good fit with $n=4$. This suggests that the conversion of hemichrome from metHb corresponding to $k_{2,obs}$

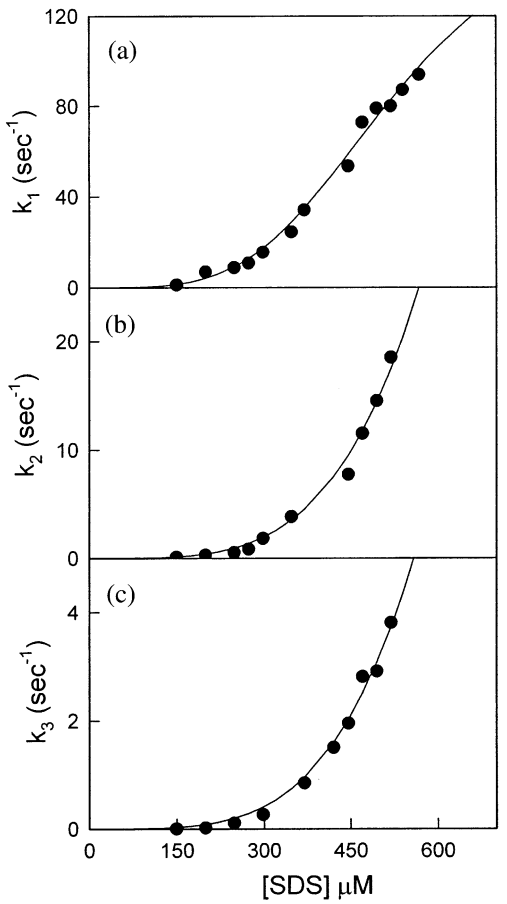

Fig. 5. (a) Plot of $k_{1,obs}$ vs. [SDS] at pH 7.0 shows for human cross-linked methemoglobin. The solid line drawn through the experimental data is the computer fit to Eq. (1). (b) Plot of $k_{2,obs}$ vs. [SDS]. (c) Plot of $k_{3,obs}$ vs. [SDS]. The solid lines drawn through the experimental data of (b,c) show the computer fit to Eq. (2).

and $k_{3,obs}$ involves irreversible interaction of the four molecules of SDS with one molecule of metHb. The kinetic data presented here show that there might be four binding sites for SDS molecules. Hemoglobin exists as a tetramer with two alphas and two beta subunits. Each of the subunits contains one heme group. The binding of four SDS molecules to one molecule of metHb suggests that each SDS molecule might be interacting with one heme group.

We have studied the kinetics of the formation of hemichrome from human cross-linked hemoglobin at 537 nm. The kinetic traces could be best fitted to three exponential functions. The variation of the rate constants with SDS concentration shows a similar behavior as observed in the case of human native metHb (Fig. 5). This suggests that covalent modification of the two alpha subunits of cross-linked hemoglobin by a fumaryl group does not seem to affect the interaction of SDS with hemoglobin. This further suggests that the SDS binding site may be away from the covalent modification. Bacterial endotoxin (LPS) which converts human native and cross-linked oxy and met hemoglobin to hemichrome, has been found to form a 1:1 complex with oxyHb [1].

3.3. Formation of superoxide radical

It is known that auto-oxidation of oxyhemoglobin is accompanied by the formation of H_2O_2 and superoxide radical, $O_2^{-\bullet}$ [7,8], and that the interaction of free fatty acids with oxyhemoglobin leads to the formation of a superoxide radical [2]. The superoxide radical is known to reduce cytochrome *c* [2]. The reduction of cytochrome *c* has often been used as a method to detect the formation of

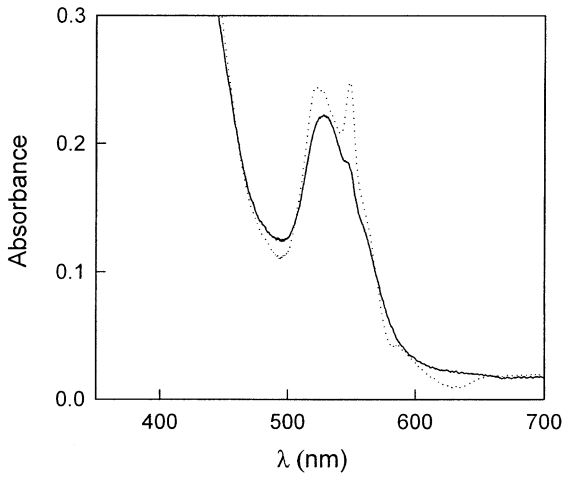

Fig. 6. Optical spectra of cytochrome *c* (solid line) and cytochrome *c* plus hemoglobin and SDS (dotted line) in 100 mM sodium phosphate buffer (pH 7.0). Hemoglobin has been taken as a baseline with 100 mM sodium phosphate buffer. The concentrations of cytochrome *c*, hemoglobin and SDS were 20, 16.7 and 100 μM, respectively.

a superoxide radical in the solution [2]. We have investigated the formation of hemichrome from oxyhemoglobin in the presence of cytochrome *c*. The optical spectrum of cytochrome *c* in the presence of oxyHb and SDS (Fig. 6) shows an increase in the reduction of cytochrome *c*. This observation is a clear indication of the formation of a superoxide radical in this reaction. It may be noted that the optical spectrum of cytochrome *c* does not show any change in the wavelength region 450–700 nm in the presence of SDS or in the presence of oxyHb without SDS (figure not shown). In oxyhemoglobin, proximal histidine and the oxygen molecule occupy the fifth and sixth positions, respectively. The distal histidine is approximately 4.2 Å away from the heme group. As discussed earlier, hemichrome is the oxidized product of hemoglobin with two histidines (proximal and distal) ligated to the six coordinated low spin irons (Fe^{3+}). The binding of SDS probably involves the interaction of both the hydrophobic tail as well as the polar head of the surfactant molecule with the protein. The heme cavity in hemoglobin is stabilized by a unique balance of hydrophobic, hydrogen bonding and polar interactions. SDS molecules probably penetrate inside the hydrophobic cavity of the heme and disturb the hydrophobic balance there. This probably triggers the removal of coordinated water and movement of distal histidine to the heme in hemichrome. In the case of MbNO, it has been found that the micellar form of SDS has a more drastic effect which leads to the depletion of the heme-NO moiety from the protein [9]. It is also suggested that the presence of the charge on the surface of the micelles may also be necessary for such processes. The formation of superoxide radical in the conversion reaction of oxyHb to hemichrome by SDS suggests that the presence of SDS induces a conformational change in the heme pocket which may induce the binding of distal histidine to the heme group forming hemichrome. The breakage of Fe–O (O2) bond in this process may lead to the formation of a superoxide radical. However, bacterial endotoxin (LPS) converts oxyhemoglobin to hemichrome without creation of any superoxide radical [1]. This suggests that the mechanism of the oxidation of oxyHb in the two cases might be different.

4. Conclusions

We observe that SDS interacts with human native and cross-linked oxyHb and metHb, and converts to low spin (Fe^{3+}) hemichrome. The conversion reaction with oxyHb leads to the formation of a superoxide radical. This suggests that SDS probably penetrates to the heme pocket and induces a conformational change, which may induce the binding of the distal histidine to iron leading to the cleavage of the Fe–O (O2) bond. The transient kinetics of the formation of hemichrome from metHb by SDS show that four molecules of SDS bind to one molecule of metHb. However, the formation of hemichrome by the interaction of bacterial endotoxin with oxyHb involves only 1:1 stoichiometry without any release of a superoxide radical. It therefore appears that the mechanism of the formation of hemichrome in the two cases is different.

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