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Interactions of anionic surfactants with methemoglobin

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ABSTRACT

Interactions of two anionic surfactants, sodium dodecyl sulphate (SDS) and sodium bis(2-ethylhexyl) sulfosuccinate (AOT) at concentrations below and above critical micelle concentration with methemoglobin (metHb) have been investigated by conventional as well as by stopped-flow absorption and fluorescence spectroscopy. The absorption spectra of metHb in AOT reverse micelles have been also analyzed. Both surfactants in their monomeric form convert metHb to reversible hemichrome. This is connected with a diminution of peroxidase-like activity of metHb and with an increase of the susceptibility of heme for a damage by H_2O_2. In micellar solutions of AOT and SDS as well as in AOT reverse micelles pentacoordinated ferric species seems to be the predominant form of this protein. It has been concluded, basing on a kinetic analysis, that conformational changes in the heme environment of metHb as induced by both surfactants occur independently of the alterations in the tertiary structure of this protein.

1. Introduction

Hemoglobin (Hb) is the major heme protein of the red blood cells responsible for the transport of oxygen to the tissues. This function of Hb is possible only when iron is in the ferrous state. The most common ferric form of hemoglobin is a methemoglobin (metHb) with water or hydroxide as a sixth coordination ligand to the iron. The amount of metHb in normal blood cells does not exceed 3%, but it can increase during the interaction of Hb with some molecules ([1], and references therein). Under some conditions exogeneous ligands of Hb and metHb are replaced by the endogeneous side chain histidine and a low-spin hemichrome is formed [2]. In vivo hemichromes are involved in Heinz bodies formation and in the elimination of the older, less functional erythrocytes [2]. Mammalian hemoglobins show a low level of hemichrome in physiological conditions at room temperature [3]. The formation of hemichromes induced by several factors may proceed without or with disruption of the native conformation. Increasing the pressure, lowering the temperature or oxygenation of NO adducts facilitate the formation of reversible hemichrome [2,3]. Irreversible hemichrome formation has been postulated to take place under denaturing conditions, e.g. addition of surfactants or dehydration [2], although partially reversible conversion of metHb to hemichrome has been observed in the presence of bacterial lipopolisaccharide (LPS) containing long hydrophobic chain of fatty acids [4], deoxycholate or ursodeoxycholate [5].

The widespread use of surfactants in analytical biochemistry has stimulated interest in the surfactant–protein interactions. The interactions of ionic surfactants, sodium dodecyl sulphate (SDS), hexadecyl trimethyl ammonium bromide (CTAB), dodecyl trimethylammonium bromide (DTAB), dodecylammonium α-glutamate (GDA) and glutamic acid-based gemini surfactants with hemoglobin (including giant extracellular hemoglobin) and methemoglobin have been studied [6–12]. The interaction between natural lipopeptide surfactant, C15, with Hb has been also reported [13]. It has been shown that ionic surfactants below critical micelle concentration (cmc) enhance the autooxidation of oxyhemoglobin to metHb followed by the formation of hemichrome [6,7,9]. On the other hand some authors have reported that autooxidation process is inhibited by SDS for [SDS]/[Hb] ratio below 50 [14] and metHb is reduced to deoxyhemoglobin when [SDS]/[Hb] ratio is below 10 [15]. The kinetics of hemichrome formation in the reaction of metHb with SDS at concentrations below cmc has been also studied [7]. The results reported in this work extend the earlier investigations concerning interactions of metHb with anionic surfactants. Sodium bis(2-ethylhexyl) sulfosuccinate (AOT) and SDS are used by us. Some results concerning the interaction of metHb with a nonionic surfactant 2-(dodecyloxy)ethanol (Brij35) are also shown for comparison.

2. Materials and methods

Hemoglobin from bovine blood was from Sigma. Methemoglobin was prepared by oxidation of Hb with a 2-fold excess of

Abbreviations: AOT, sodium bis(2-ethylhexyl) sulfosuccinate; Brij35, 2-(dodecyloxy)ethanol; cmc, critical micelle concentration; CTAB, hexadecyl trimethyl ammonium bromide; DTAB, dodecyl trimethylammonium bromide; GDA, *n*-dodecylammonium α-glutamate; Hb, hemoglobin; LPS, bacterial lipopolisaccharide; Mb, myoglobin; metHb, methemoglobin; SDS, sodium dodecyl sulphate.

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potassium ferricyanide and passage through a column containing Sephadex G-25 using 10 mM phosphate buffer, pH 7.0, as an eluent. Concentration of metHb was determined spectrophotometrically using $\varepsilon_{405} = 1.79 \times 10^5\ M^{-1}\ cm^{-1}$, expressed per heme [16].

AOT, SDS and Brij35 were from Sigma. AOT and SDS were dried under vacuum over P_2O_5. AOT reverse micelles were formed by injection of appropriate amounts of aqueous stock solutions in phosphate buffer, into 0.1 M AOT in *n*-heptane (Fluka) to obtain the desired w_o, i.e. the ratio of $[H_2O]$ to [AOT], where water indicates the buffer alone or buffer containing metHb. A mixture was shaken until a completely transparent solution was obtained. All measurements were done in 10 mM phosphate buffer. Water from MilliQ Plus was used. Measurements were done at ambient temperature 24 ± 1 °C. Cmc in 10 mM phosphate buffer determined by a conductometric method was 2.5 and 4.2 mM for AOT and SDS, respectively.

Rapid absorption changes of metHb were studied on an Applied Photophysics SX 18MV stopped-flow spectrofluorimeter with 1 cm cell. A Hewlett–Packard 8452A diode-array spectrophotometer was used for absorption measurements in a long-time regime. Fluorescence measurements were also carried out on an Applied Photophysics stopped-flow apparatus working in a fluorescence mode (0.2 cm cell). The excitation wavelength was 280 nm and a wideband interference filter (320–450 nm) was used.

3. Results

It is known that absorption spectra of heme proteins are influenced by heme microenvironment. Methemoglobin, at neutral pH, possesses a characteristic absorption spectrum peaking at 406 nm (Soret), around 500 nm (Q) and at 630 nm (ligand-to-metal charge transfer, LMCT). We found that the absorption spectra in the Soret region of 2.5 μM metHb in the presence of AOT at concentrations below 4×10^{-5} M, at pH 7.0, slightly decreased without any shift of an absorption maximum. The shift of the Soret band of metHb to 408 nm took place at 4×10^{-5} M of AOT. In the presence of 0.2–1 mM of AOT the absorption spectrum in the Soret region was characteristic for hemichrome (414 nm). Similar observations have been made after adding metHb and metmyoglobin (metMb) to SDS, CTAB, DTAB or GDA solutions at concentrations below cmc [7,8,10,12,17–19]. The absorption spectra of hemoglobin species in the presence of AOT at concentration ≥2 mM shifted towards shorter wavelengths with the concomitant decrease of an absorbance to about one half of the initial metHb absorbance (Fig. 1). A blue shift of the Soret band from 414 to 406 nm is related to the formation of pentacoordinated species [20–22]. The broad and asymmetric Soret band may indicate the coexistence of two high-spin pentacoordinated species, i.e. a species with proximal histidine as fifth ligand and a pentacoordinated free heme with water molecule as fifth ligand, without direct contact with polypeptide chains (in other words, heme is dissociated from the protein) [20–22]. In Table 1, we compared the wavelengths of the absorption maxima of hemoglobin species in the Soret region in the presence of AOT and SDS. The absorption spectra of hemoglobin species in the presence of AOT taken in the visible region are shown in Fig. 2. The band at 534 nm and a shoulder around 565 nm, characteristic for hemichrome [23], appeared in the presence of AOT at concentration range from 0.2 to 3 mM, i.e. for the ratio [AOT]/[metHb] from 10 to 125 (initial metHb concentration was 20 μM). Unfortunately, we were not able to mix 20 μM metHb with AOT at a concentration higher than 3 mM. In the case of SDS we observed hemichrome formation in the visible absorption spectra of hemoglobin species when [SDS]/[metHb] ≥7 (data not shown). The band at 630 nm disappears when a low-spin hemichrome is formed (Fig. 2). An increase of the LMCT band observed in the presence of 3 mM AOT (line 5) indicates the formation of pentacoordinated high-spin species. Similar observation has been made for metHb in the presence of SDS or DTAB at concentrations higher than cmc [10].

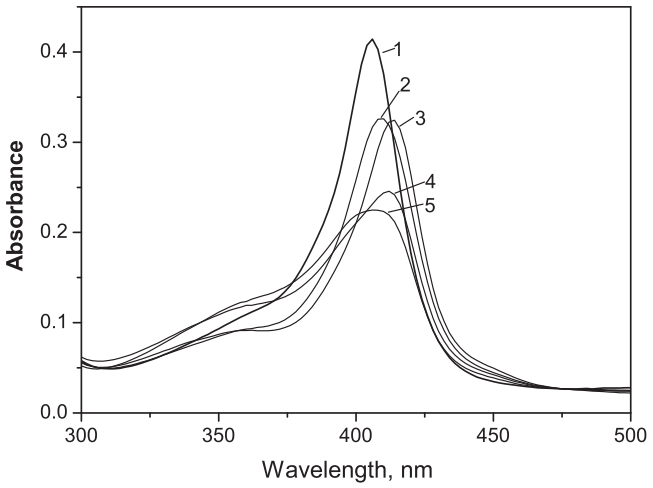

Fig. 1. Absorption spectra in the Soret region of 2.5 μM metHb in a buffer solution (1) and in the presence of 0.1 mM (2), 1 mM (3), 2 mM (4) and 3 mM (5) AOT.

Table 1
The wavelengths of the absorption maxima of hemoglobin species at the Soret region in the presence of AOT and SDS. [metHb] = 2.5 μM.

[Surfactant], mM	λ_{max} in the presence of AOT	λ_{max} in the presence of SDS
0.01	406	406
0.02	406	408
0.04	408	–
0.05	410	410
0.1	410	412
0.15	412	414
0.2	414	414
0.4	414	414
0.6	414	414
1	414	414
2	412	412
2.5	412	412
3	–	412
4	410	412
5	408	406
7	406	404

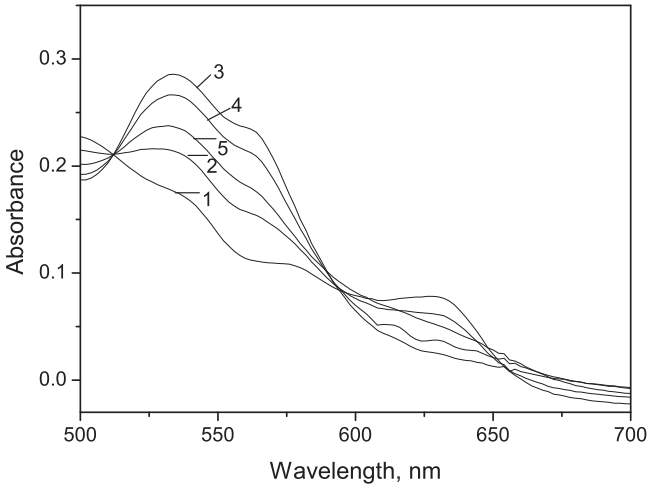

Fig. 2. Absorption spectra in the visible region of 20 μM metHb in a buffer solution (1) and in the presence of 0.1 mM (2), 1 mM (3), 2 mM (4) and 3 mM (5) AOT.