Biochemistry and Biophysics Reports 7 (2016) 386–393

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Sulfmyoglobin conformational change: A role in the decrease of oxy-myoglobin functionality

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ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form
28 June 2016
Accepted 1 July 2016
Available online 7 July 2016

Keywords:
Sulfmyoglobin (SMb)
Myoglobin (Mb)
Hemoglobin I (HbI)
Hydrogen sulfide (H_2S)
SAXS
WAXS

ABSTRACT

This work is focused at understanding the interaction of H_2S with Myoglobin (Mb), in particular the Sulfmyoglobin (SMb) product, whose physiological role is controversial and not well understood. The scattering curves, Guinier, Kratky, Porod and *P(r)* plots were analyzed for oxy-Mb and oxy-Hemoglobin I (oxyHbI) in the absence and presence of H_2S, using Small and Wide Angle X-ray Scattering (SAXS/WAXS) technique. Three dimensional models were also generated from the SAXS/WAXS data. The results show that SMb formation, produced by oxyMb and H_2S interaction, induces a change in the protein conformation where its envelope has a very small cleft and the protein is more flexible, less rigid and compact. Based on the direct relationship between Mb's structural conformation and its functionality, we suggest that the conformational change observed upon SMb formation plays a contribution to the protein decrease in O_2 affinity and, therefore, on its functionality.

1. Introduction

Hydrogen Sulfide (H_2S) is a gas with a "rotten egg" like smell produced in different systems in nature and industrial processes [1,2]. Through the years, H_2S was known as a toxic gas that disrupts the respiratory process by inhibition of the cytochrome c oxidase (CcO) [1–5]. It also forms sulfhemoglobinemia, a rare blood condition with anemic/cyanotic symptoms, induced by the increase concentration of Sulfmyoglobin (SMb) and Sulfhemoglobin (SHb) complexes [5–9]. However, another side of the H_2S molecule arose when it was discovered that there were three proteins [cysthathionine β-synthase (CBS), cysthathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (MST)] that produced H_2S physiologically and were present in different parts of the human body [1–5]. Different cytoprotective roles have been associated to H_2S in the respiratory, vascular, nervous, endocrine and gastrointestinal systems [1–5]. For this reason, H_2S has been evaluated as a potential therapeutic tool for the treatment of multiple diseases [10,11]. Nevertheless, the concept of H_2S therapy should be treated with caution since there is a thin line between its cytoprotective and cytotoxic aspects defined by concentration. The H_2S physiological concentration has been suggested to be low, ranging from nM to μM [1–3]. When a person is exposed to high H_2S concentration, there is an increase in physiological H_2S concentration that leads to the cytotoxic effects dominating over cytoprotective [2]. This is important when developing an efficient therapeutic tool, where the beneficial effects are maximized and the harmful effects minimized. The process for developing a resourceful H_2S therapy requires a better understanding of the chemistry of H_2S with different systems, in particular, protein interaction with this molecule.

Our research is focused at understanding one of the products of the interaction of H_2S with hemeproteins, in particular the sulfheme proteins (SMb and SHb), whose physiological role is controversial and not well understood. When myoglobin (Mb) and hemoglobin (Hb) are exposed to H_2S in the presence of oxygen (O_2) or hydrogen peroxide (H_2O_2), a sulfur atom incorporates across the β-β double bond of the pyrrole B, as shown in Fig. 1 [7,8]. This sulfur ring formation can be identified by its characteristic absorption bands around 620 or 715 nm, depending on the bound-ligand and oxidation state of the heme-iron [7]. Resonance Raman is another tool for the recognition of the sulfheme complex formation by evaluation of the vinyl modes bands (1620 and 1026 cm^{-1}) and the satellites bands around υ_4 [7,8]. Moreover, the presence of a properly oriented distal His residue is crucial for the sulfheme complex formation. This was determined by analyzing the H_2S reactive Hemoglobin I (HbI) from the clam *Lucina pectinata* that interestingly does not form the sulfheme complex, given that it lacks of a distal His residue [7,8]. When the sulfheme complex forms, Mb decreases its O_2 affinity by approximately 2500 folds. This significant decrease in O_2 affinity was

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http://dx.doi.org/10.1016/j.bbrep.2016.07.002

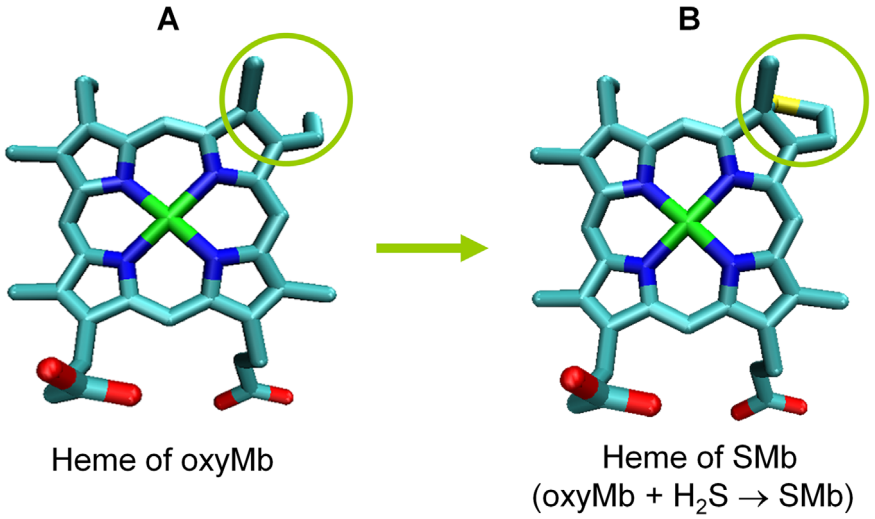

Fig. 1. Sulfmyoglobin (SMb) Structure. Myoglobin (A, left) and SMb isomer C (B, right); PDB files 1MBO (Mb) and 1YMC (SMb).

determined by comparing the 50% oxygenation values in the oxygen binding curve of Mb and SMb (0.79 and 0.00028 atm, respectively) [12]. Berzofsky et al. determined the local chemical change that plays a part in the decrease of the protein functionality. They evaluated the bond strength of the heme Fe-ligand of Mb and SMb by IR studies. A 10 cm^{-1} red-shift was observed in the IR spectra of the SMb complex, representative of a decrease in electron density, bond order and strength of the heme-ligand interaction. They attributed this to the electron withdrawing effect of the sulfur atom in the heme macrocycle, resulting in a decrease in electron density of the Fe. As a consequence, the heme-Fe^{II} acquired a character of heme-Fe^{III}; thus, weakening the Fe-ligand bond since the metal-ligand π contribution is compromised. This is also favored by the rupture of the heme group conjugation produced by the heme distortion, as result of the sulfur ring incorporation [12,13]. Moreover, Berzofsky et al. suggested that a 0.5 cm^{-1} shift corresponds to a decrease in O_2 affinity by a factor of 10, which indicates that this local chemical structural change contributes to only a 200 fold decrease in O_2 affinity [12], representing only 8% of the total decrease in affinity (2500 folds) determined by the oxygen binding curve. Therefore, there are other significant changes occurring in the protein as a consequence of the sulfheme complex formation that also contribute to the decrease in protein functionality.

2. Material and methods

2.1. Sample preparation

Myoglobin (Mb) from equine heart was purchased from Sigma-Aldrich. Recombinant HbI (rHbI) was prepared and purified as previously reported [23] and used as control. Both proteins were dissolved in a 100 mM Succinic Acid, 100 mM Potassium dihydrogen phosphate, and 1 mM EDTA buffer, 6.5 pH (all purchased in Sigma-Aldrich). The oxy-derivatives were prepared by adding [1:15] concentration ratio of [protein: sodium dithionite] under anaerobic conditions followed by O_2 purging [4]. The H_2S solution was prepared by dissolving sodium sulfide (purchased in Alfa-Aesar) in the previously mention anaerobic buffer. The sulfheme complex formation was monitored through its characteristic 620 nm band by UV–vis spectroscopy using an Agilent 8453 spectrophotometer [8]. The sulfheme complex was acquired by adding H_2S to the oxyMb complex in a [1:70] concentration ratio of [oxyMb: H_2S] that provides the highest intensity and stability of the 620 nm characteristic band.

2.2. SAXS/WAXS data acquisition and processing

SAXS/WAXS data were recorded on oxyMb and oxyHbI in the absence and presence of H_2S. The 620 nm band was monitored before and after data acquisition for both proteins. The band was only detected in oxyMb after addition of H_2S since oxyHbI does not form the sulfheme derivative. The 100 mM succinic acid, 100 mM potassium dihydrogen phosphate, and 1 mM EDTA buffer was used as a background. In the absence of H_2S, the scattering data of oxyMb were first collected at 5, 6, 10, and 11 mg/mL. The optimal protein concentration was found to be 11 mg/mL and the scattering data in the presence of H_2S were therefore collected at this concentration. For HbI the final protein concentration was 6.7 mg/mL. The SAXS/WAXS data was collected simultaneously at the X-9 Beamline of the National Synchrotron Light Source at Brookhaven National Laboratory using a PILATUS 300k SAXS detector and a Photonic Science CCD WAXS detector [24]. For triplicated data acquisition, 20 μL of sample was continuously flowed through a 1-mm diameter capillary where it was exposed to the x-ray beam for 30 s. Initial data processing was performed using the pyXS-v2 software package developed at X9. The program converted the two-dimensional scattering patterns recorded on the SAXS/WAXS detectors into one-dimensional scattering profiles. Three scattering patterns of each sample were obtained, averaged, and buffer (background) subtracted. Further SAXS/WAXS data processing and analysis were performed using 2.5.2 ATSAS Package [25,26]. Guinier, Kratky and Porod [20] analyses were conducted using Primus [27] The pair distribution functions were evaluated using GNOM [28]. 3-D surfaces were generated using DAMMIN [29], averaged using DAMAVER [30], and superimposed using SUPCOMB [31]. The theoretical scattering profiles of oxyMb and SMb atomic models were evaluated using CRYSOL [32]. Pymol was used for graphical visualization and figure generation.

3. Results and discussion

Characterization of oxyMb and oxyHbI with and without H_2S was conducted using their overall dimensions and shapes, as well as their internal structural features derived from SAXS and WAXS scattering data, respectively.

3.1. Scattering curve and Guinier plot

In SAXS/WAXS, the intensity of the scattered X-ray beam is