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Structural determinants for the formation of sulfhemeprotein complexes

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Abstract

Several hemoglobins were explored by UV-Vis and resonance Raman spectroscopy to define sulfheme complex formation. Evaluation of these proteins upon the reaction with H₂O₂ or O₂ in the presence of H₂S suggest: (a) the formation of the sulfheme derivate requires a HisE7 residue in the heme distal site with an adequate orientation to form an active ternary complex; (b) that the ternary complex intermediate involves the HisE7, the peroxo or ferryl species, and the H₂S molecule. This moiety precedes and triggers the sulfheme formation.

Keywords

sulfhemoglobin; sulfmyoglobin; hemoglobin I (HbI); hydrogen sulfide (H₂S); hydrogen peroxide (H₂O₂); histidine (His); ferryl species

Introduction

Historically, hydrogen sulfide (H₂S) has been regarded as a poisonous gas with a wide spectrum of cytotoxic effects [1]. However, a new controversial role is emerging for H₂S in the chemistry of biological systems. It has been found that H₂S is synthesized endogenously in mammalian tissues and that it functions as a neuromodulator, and a smooth muscle relaxant [2,3]. Furthermore, it has been suggested that the interaction of H₂S with cytochrome *c* oxidase, hemoglobin (Hb) and/or myoglobin (Mb), can decrease cellular ATP, activating the K_{ATP} channels, and causing in turn hyperpolarization (neurons) or smooth muscle relaxation [4,5].

In this respect, the reaction of H₂S with Hb and Mb, in the presence of H₂O₂ or O₂, results in covalent modification of the heme pyrrole ring bearing the 4-vinyl group, generating the

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so-called sulfmyoglobin and sulfhemoglobin derivatives [6,9]. The suggested structure has been described as a chlorin with H₂S added only across the β - β double bond of the pyrrole “B” [7–9]. These sulfheme derivatives have lower O₂ affinity, which can in principle activate the K_{ATP} channels [5]. Increased levels of sulfhemoglobin in humans can lead to rare cases of the blood disease sulfhemoglobinemia, treatments for which remain unreliable [10–12] since the mechanism underlying sulfheme complex formation has not been elucidated. The sulfheme complex has a characteristic absorption band at 620 nm that is probably due to the distortion of heme group planarity and alterations within of neighboring side-chains relative to the native protein [7–9]. It has been suggested though, that the interaction of Hb (or Mb) with H₂O₂ produces ferryl Compound I, (Fe^{IV}=O Por^{•+}) and ferryl Compound II (Fe^{IV}=O Por) [13], and that H₂S may interact with these species to produce the sulfheme derivative [7,8]. However, other hemeproteins, including the hemoglobins from the clam *Lucina pectinata* (HbI, HbII and HbIII), also form these ferryl species in the presence of H₂S without generating the sulfheme derivatives [13–14].

The clam *Lucina pectinata* has three peculiar hemoglobins with distinctive chemical-physical properties [14]. The monomeric HbI binds and transports H₂S while HbII and HbIII, are O₂ reactive. The proposed mechanism suggests that HbI reacts with H₂S in the presence of O₂ to form a hemeFe(III)-H₂S low spin complex. It is noteworthy to mention that in the presence of H₂O₂ and H₂S, HbI forms stable Compound I species without generating the sulfheme derivative [13]. It has also been reported that neither HbII nor HbIII, which produce stable Compound II, form the sulfheme product. Interestingly, HbI, HbII, and HbIII contain a distinctive distal structural organization involving a GlnE7 residue instead of the typical His found in Hb and Mb [15,16].

Therefore, it is plausible to hypothesize that the distal site environment may play a crucial role in sulfheme production since the reactivity of H₂S with hemeproteins depends on the H₂S concentration and the polarity of the distal heme cavity [17]. To confirm the role of the distal residues on sulfheme formation, we have evaluated the interaction of Mb, human Hb, HbI, HbII/III, several HbI mutants and the hemoglobins from the *Macrobdella* and *Lumbricus*, with H₂O₂ and H₂S. Specifically, single point mutations were introduced into the *Lucina* HbI heme pocket to mimic the Mb distal site and to identify the residues involved in sulfheme formation. Here, we report unprecedented results of a new role for HisE7, in the presence of H₂S and H₂O₂ or O₂, essential for the formation of the sulfhemeprotein derivative.

Material and Methods

Sperm whale Mb and human Hb were purchased from Sigma-Aldrich. Native HbI and HbII/HbIII proteins, as well as the HbI mutants were prepared and purified as previously reported [14,18–19]. The purified hemoglobins from *Macrobdella* and *Lumbricus*, were provided by Dr. Serge Vinogradov. The sulfheme samples were prepared by mixing solutions of the ferric hemoglobins with solutions of H₂O₂ and H₂S [17] in a ratio of 1:2:20 (Mb:H₂O₂:H₂S). The samples were then equilibrated and the optical spectra were recorded using an Agilent 8453 spectrophotometer. The resonance Raman (RR) spectra were obtained as described before [17].

Results and Discussion

Myoglobin was used as the control since the properties of the sulfmyoglobin have been described in several reports [6–9]. Figure 1A shows the UV-Vis spectra obtained upon reacting metMb, metHb, metHbI and metHbI GlnE7His with H₂O₂ and H₂S. Formations of the sulfmyoglobin and sulfhemoglobin derivatives are clearly demonstrated by the presence of

the characteristic sulfheme Q band at 618 and 620nm, respectively. Remarkably, HbI did not form the sulfheme derivate, as judged by the absence of a defined band near 620nm. On the other hand, when Gln is replaced by His in HbI GlnE7His mutant, a distinctive band at 622nm was observed that noticeably indicates the existence of the sulfheme complex. Indeed, Figure 1B shows that this band is due the formation of sulfheme derivative and not to a modification of the heme induced by H₂O₂ itself. It is well known that the heme may become oxidatively modified when it interacts with H₂O₂ [20]. However, modification of the heme by H₂O₂ can be discarded since the alteration of the heme, observed by the formation of the characteristic 620 sulfheme band, occurs only after the addition of H₂S. Therefore, the modification of the heme in all the proteins studied here can be attributed to the interaction of H₂S. Similar reactions were observed for the analogous oxyheme complexes and H₂S [7].

In general, Table 1 summarizes the spectrophotometric results obtained with the proteins evaluated for sulfheme formation, together with their distal site amino acid residues. The only proteins that exhibit the band near 620nm, were the ones that have His in the E7 position, as in horse heart Mb, human Hb, and the HbI GlnE7His mutant. This common feature indicates that the distal His is crucial for sulfheme complex formation. Moreover, the fact that no sulfheme complex was observed for the HbI PheB10His mutant, which has a His residue in its active site, albeit not in the same orientation towards the heme group as the distal His, strongly suggests that the orientation of the His is also fundamental for sulfheme formation. The sulfheme formation was also observed in the giant hemoglobins from *Macrobdella* which bear His at position E7 (see Figure 1B insert). The variety of hemeproteins that produce the sulfheme complex, ranging from the giant, multi-chain, extracellular hemoglobins from *Macrobdella* and *Lumbricus* [21,22] to the monomeric Mb and the *Lucina* HbI GlnE7His mutant strongly indicates that the formation of the sulfheme derivative requires an intermediate ternary complex involving His E7, a peroxo or ferryl species and H₂S. Other protein changes, although present and necessary, are not unique for the formation of the sulf heme derivative, suggesting that the ternary complex intermediate is the pathway for the formation of the sulfhemeprotein species. It is appropriate to mention that no sulfheme formation was observed in a recent study of sulfide binding by group 2 2/2 bacterial Hbs, in line with the absence of distal E7His residues [23] and therefore, lacking the ternary complex intermediate necessary for the sulfheme formation.

The intensity of the 620 nm band (Figure 1A and 1B) is different in each of the sulfheme proteins evaluated. This band arises from the forbidden electronic transition in the visible or Q region of the spectra, which becomes partially allowed due to configuration interactions between the heme chlorin orbitals. Distortion of the heme can increase or decrease the energy of heme orbitals [24], thus affecting the intensity of the band associated with the forbidden transition. In this regard, heme planarity distortion increases these interactions, increasing in turn the intensity of the 620 nm band. It is likely that the observed variation in the intensities of the 620 nm band in the sulfheme proteins studied here can be accounted for by variation in the distortion of heme planarity.

To further demonstrate that the presence and orientation of His is vital for sulfheme formation, an analysis of the porphyrin distortion and alteration in the conformation of the vinyl groups was undertaken using. The first RR evidence indicating sulfheme complex formation in Mb and the *Lucina* HbI GlnE7His mutant, was the observation of an increase in the number and intensity of the RR bands (Figure not shown), which was not observed in HbI. In the sulfheme complex, alteration of the porphyrin macrocycle from D_{4h} symmetry to a less symmetric chlorin C₂ heme leads to a distortion of the macrocycle planarity and changes in the heme electronic properties, resulting in the activation of several out-of-plane heme modes [25].

Moreover, as Figure 2A indicates that the resonance Raman spectra of Mb and the HbI GlnE7His mutant, in the presence of H₂O₂ and H₂S, show a substantial increase in the intensity of the modes at 1353 and 1390cm⁻¹, which are absent in their metaquo-heme complexes and in the HbI protein. The development of these bands in the Mb and HbI GlnE7His spectra, and not in the HbI spectrum, indicates a distortion of the heme group due to the incorporation of the sulfur ring [25]. This suggestion is supported by the fact that the 1353 and 1390cm⁻¹ peaks have been related to the B_{1g} pyrrole deformation of chlorin hemes [25,27].

Sulfheme formation in Mb and the HbI GlnE7His mutant was also confirmed by evaluating the vinyl bands (νC=C) at ~1620 cm⁻¹ [26,27], in the RR spectra of Mb, HbI and HbI GlnE7His, with and without H₂O₂ and H₂S, as shown in Figure 2B. The Figure clearly shows that in the absence of H₂O₂ and H₂S, the vinyl modes can be deconvoluted into two distinct bands at 1620 and 1626 cm⁻¹. Upon the exposure of the hemeprotein samples were exposed to H₂O₂ and H₂S, a substantial reduction of the 1626 cm⁻¹ vinyl band was observed for Mb and the HbI GlnE7His mutant, while no change was observed for the HbI protein. The reduction of the 1626 cm⁻¹ band is associated with a decrease in conjugation of the system induced by the incorporation of the characteristic sulf ring, as shown in the insert of Figure 2A [25]. In this regard, the chlorin heme is a less conjugated system than the porphyrin macrocycle, which agrees with the observed decrease in the 1626 cm⁻¹ band intensity. Curiously, the absence of these bands in the *Lucina* Hbs with the distal GlnE7 is in agreement with the conclusion that the decrease in conjugation and the formation of new bands are strongly linked to the formation of the chlorin derivative characteristic of the sulfheme complex. Formation of the latter requires the presence of the distal HisE7 in the heme active site.

Furthermore, we propose here that the peroxo or the ferryl species involved in sulfheme formation are the ones with which His has a direct interaction, and that the H₂S proton interaction with the His-ferryl (or peroxo)-heme ternary complex triggers the formation of the sulfheme complex. This proposal is supported by the fact that formation of sulfheme was only observed with proton donor thiols such as methanethiol (CH₃SH), hydrogen sulfide (HSH) and cysteine (R-CH₂SH), while no sulfheme was detected with the dimethyl sulfide (CH₃SCH₃) molecule (Figure not shown). Thus, these results suggest that the proton form the HSR group plays also a significant role in the formation with heme ferryl ternary complex triggering the formation of the sulfheme derivatives.

Conclusion

In summary, we propose that the generation of the sulfheme derivative requires the presence of peroxo or ferryl species and a distal HisE7 residue properly oriented to form the active ternary complex. Our results clearly indicate the direction to be taken in further investigations of the mechanism for sulfheme protein complex formation.

Research Highlights: “Structural determinants for the formation of sulfhemeprotein complexes”

- HisE7 is crucial for the sulfheme formation
- HisE7 needs an appropriate orientation for an adequate interaction
- the ternary intermediate involves HisE7, peroxo or ferryl species, and H₂S molecule
- this moiety precedes and triggers the sulfheme formation

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Figure 1.

UV-Vis spectra of Mb, human Hb, HbI, the HbI GlnE7His mutant and *Macrobdella* Hb.

Figure 1A. Optical spectra of metMb (dash line), met human Hb (dotted-dashed line), methHbI (dotted line), and HbI GlnE7His (solid line) in the presence of H₂O₂ and H₂S.

Figure 1B. Optical spectra of the reaction sequence of methHbI GlnE7His (dotted line) with H₂O₂ (dashed line) and H₂S (solid line). The inserted optical spectra of *Macrobdella* Hb with H₂O₂ (dashed line) and H₂S (solid line).

Figure 2.

Resonance Raman spectra of Mb, HbI, and the HbI GlnE7His mutant. Figure 2A shows the adjacent modes to ν_4 of metMb (dashed line), metHbI (dotted line), and HbI GlnE7His (solid line), in the presence of H_2O_2 and H_2S . Figure 2B illustrates the decomposition of the vinyl bands. The left panel shows the met complexes while the right panel demonstrates heme proteins in the presence of H_2O_2 and H_2S . The filled squared lines are the fit after deconvolution.

Table 1

Sulfheme formation in the heme proteins used in this study and their distal active site residues. Note that each *Macrobdella* and *Lumbricus* Hbs have four different globin chains [21,22]. (–), not observed

Protein	Residues Position			Sulfheme Q band (nm)
	E7	B10	E11	
Horse heart wt-Mb	His	Leu	Val	618
Human wt-Hb	His	Leu	Val	620
<i>Lucina</i> wt-HbII/III	Gln	Phe	Phe	–
<i>Lucina</i> wt-HbI	Gln	Phe	Phe	–
<i>Lucina</i> PheE11Val	Gln	Phe	Val	–
<i>Lucina</i> PheB10Leu	Gln	Leu	Phe	–
<i>Lucina</i> GlnE7His	His	Phe	Phe	622
<i>Lucina</i> PheB10His	Gln	His	Phe	–
<i>Macrobdella</i> Hb	His	YFFL	LLLF	622
<i>Lumbricus</i> Hb	His	FLWW	VIVV	622