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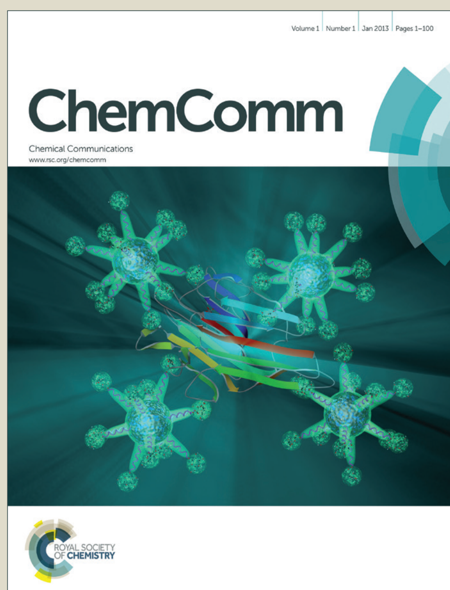

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Reaction between a haemoglobin model compound and hydrosulphide in aqueous solution†

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The reaction between hydrosulphide (SH^-) and a haemoglobin model, composed of a Fe(III)-porphyrin and a cyclodextrin dimer possessing a pyridine-linker (met-hemoCD3), was studied. Met-hemoCD3 formed a stable (SH^-)-met-hemoCD3 complex in oxygen-free neutral aqueous solution. In the presence of O_2, reversible ligand exchange between (SH^-)-met-hemoCD3 (Fe(III)) and O_2-hemoCD3 (Fe(II)) occurred.

In biological systems, hydrogen sulphide (H_2S; pK_a = 6.8) is produced by the action of several enzymes such as cystathionine β-synthase, cystathionine γ-lyase, and 3-mercaptopyruvate sulphurtransferase, which utilise L-cysteine or L-homocysteine as substrates.[1] Although the quantity of H_2S produced in the mammalian body is small (<10 ppm), H_2S plays an important role in the regulation of signal transmission.[2] The biological function of H_2S has been the focus of intense research, along with other gaseous signalling molecules such as carbon monoxide (CO) and nitric oxide (NO).[2] When a large amount of H_2S (>700 ppm) is inhaled from the environment, some of the excess H_2S is rapidly decomposed in the blood. However, when the amount of H_2S inhaled exceeds the threshold limit of blood, H_2S inhibits the enzymatic activity of cytochrome *c* oxidase (CC_{ox}), a member of the respiratory chain in the mitochondria, through binding to Fe(II) in CC_{ox}. Thus, H_2S is highly toxic for living organisms.[3] Collman et al. studied the reaction between H_2S and a synthetic CC_{ox} model, composed of a picket fence-type Fe(II)-porphyrin attached to a Cu(I)-triimidazole moiety, in buffered aqueous solution (pH 7).[4] Their results revealed that H_2S reduces Fe(III) of CC_{ox} and binds to Fe(II), therefore inhibits the catalytic activity of CC_{ox} by displacing molecular oxygen (O_2).

H_2S also binds to Fe(III) metmyoglobin (metMb) and methaemoglobin (metHb), with a binding constant (K) of 5×10^4 M^{-1}.[5] *Lucina pectinata*, a type of clam inhabiting sulphide-rich environments, expresses monomeric metHbI. This monomeric form exhibits higher binding affinity towards H_2S than mammalian metHb.[6] In metHbI, the distal histidine (His) residue of metHb is replaced by a glutamine (Glu) residue, which stabilises the H_2S complex. However, various other factors are also involved in determining the stability of the H_2S complex.[7,8] Moreover, when H_2S and hydrogen peroxide (H_2O_2), which is generated under conditions of oxidative stress, coexist, the sulphur (S) atom is inserted into haeme.[9] This sulphur insertion is proposed to be mediated by the reaction between the •SH radical and the oxo-ferryl (Fe^{IV}=O) complex, which is produced by the reaction of haeme with reactive oxygen species.[10]

Therefore, investigating the reactions between a simple haeme model compound with the defined structure and H_2S might provide insights into the complex reactivity of haemeproteins with H_2S. Several research groups have studied the reactions between synthetic porphyrins and H_2S.[11-16] However, there is much room for investigating coordination of hydrosulphide to an Hb model compound while simultaneously considering the effects of the axial ligand and the solvent.

Our group has extensively studied 1:1 inclusion complexes of a water-soluble Fe-porphyrin and methylated β-cyclodextrin dimers linked via various bridges having nitrogenous ligands (hemoCDs), which exhibit haemeprotein-like functions in aqueous solution.[17-22] Met-hemoCD3 is the 1:1 inclusion complex of meso-tetrakis(4-sulfonatophenyl)porphinatoiron(III) (Fe(III)TPPS) and Py3OCD. Py3OCD is the per-*O*-methylated β-cyclodextrin dimer linked to a OCH_2PyCH_2O (Py: pyridine-3,5-

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diyl) bridge at the 3- and 3'-positions of the glucopyranose units (ESI†).[22] The structure of met-hemoCD3 was determined by X-ray crystallography, which revealed that the Fe-porphyrin moiety is completely buried within the cyclodextrin moiety and is axially coordinated by the pyridine ligand.[22] Because the hydrophobic environment of the cyclodextrin pores surrounds the Fe-porphyrin neighbourhood, hemoCD3 forms a stable Fe(II)-O_2 complex. In general, the Fe(II)-O_2 bond is easily oxidised by the nucleophilic attack of a water molecule.[17] The hemoCDs in the ferrous states reversibly bind O_2, CO, and NO,[18,20] whereas those in the ferric states (met-hemoCDs) bind inorganic anions such as N_3^- and CN^- in aqueous solution.[18,22] In addition, met-hemoCDs possess peroxidase-like functions.[23-25] These results suggest that met-hemoCD3, a convenient haemeprotein model, binds SH^- in the same way as metHb.

In this study, NaSH was used as the source of SH^-. The electronic structure and reactivity of met-hemoCD3 in the presence of NaSH were studied by EPR. A high spin signal[26] due to met-hemoCD3 at g = 6.23 in oxygen-free phosphate buffer (pH 6) at 77 K disappeared upon addition of NaSH (1–2 eq.), and new characteristic signals at g = 2.48, 2.40, 2.26, and 1.94, ascribed to six-coordinated low-spin (SH^-)-met-hemoCD3, appeared (Fig. 1,

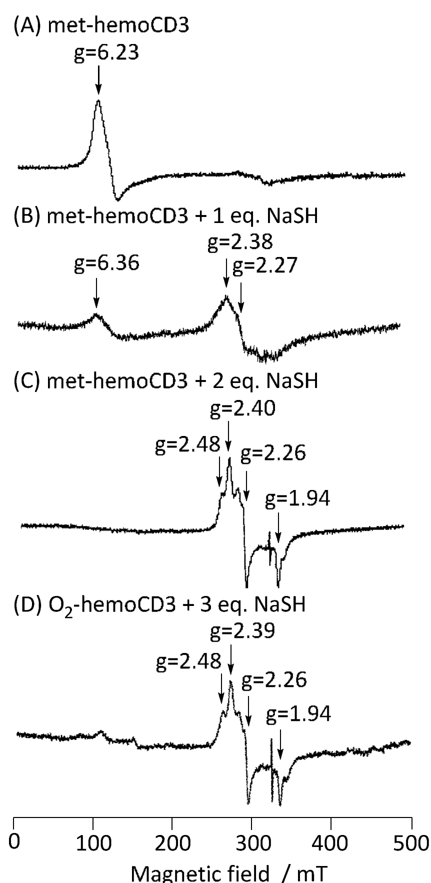


Fig. 1 EPR spectra of met-hemoCD3 (5×10^{-4} M) in the absence (A) and presence of 1 eq. (B) and 2 eq. (C) of NaSH in phosphate buffer at pH 6 and 77 K. (D) EPR spectrum of O_2-hemoCD3 (5×10^{-4} M) upon addition of 3 eq. NaSH.

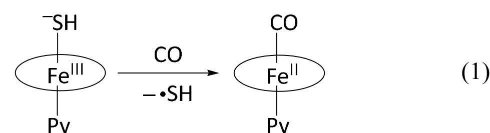
A–C). In contrast, addition of NaSH to the Fe(III)TPPS/(TMe-β-CD)$_2$ complex (Fig. S1, ESI†; TMe-β-CD: heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin), which does not possess the axial nitrogenous ligand, caused the disappearance of the EPR signals, indicating that a diamagnetic Fe(II) species was produced through the reduction of Fe(III) with SH^-. These results suggest that the axial pyridine ligand is essential for the formation of a stable Fe(III)-(SH^-) complex.

The formation constants (K) of (SH^-)-met-hemoCD3 at various pHs were determined by UV-Vis titration (Table 1; Fig. S2, ESI†). Because OH^- competes with SH^- to bind to Fe(III),

Table 1 Formation constants (K) of (SH^-)-met-hemoCD3, determined by titration at 25 °C.

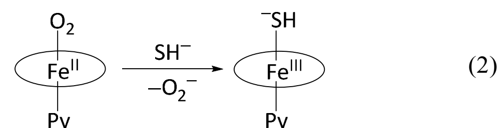
pH	K / M^{-1}
6	3.1×10^5
7	9.6×10^4
8	2.5×10^5
9	7.1×10^3
10	2.8×10^3

the K value of (SH^-)-met-hemoCD3 decreased at higher pH. Conversely, at neutral pH (pH 6–8), the K values for (SH^-)-met-hemoCD3 were considerably high (10^4–10^5 M^{-1}; Table 1). The (SH^-)-met-hemoCD3 complex shows relatively high reactivity in the reduction of the complex to Fe(II)-hemoCD3 (Fig. S3, ESI†). When CO, a strong coordinating ligand of Fe(II), was introduced into the (SH^-)-met-hemoCD3 system, rapid formation of CO-hemoCD[22] was observed (Fig. S4, ESI†). These results suggest that homolysis of the Fe–S bond occurred, followed by insertion of CO [eqn. (1)]:



(1)

Interestingly, the EPR spectrum of Fe(II)-hemoCD3 did not change upon addition of NaSH (3 eq.), while the addition of NaSH (3 eq.) to O_2-hemoCD3 (5.0×10^{-4} M) generated EPR signals at g = 2.48, 2.39, 2.26, and 1.94, ascribed to (SH^-)-met-hemoCD3 (Fig. 1D). These results indicate that SH^- does not react with Fe(II)-hemoCD3, while, in the case of O_2-hemoCD3, ligand exchange of O_2^- with SH^- and oxidation to Fe(III) occur spontaneously, resulting in the formation of (SH^-)-met-hemoCD3 [eqn. (2)]:



(2)

When a solution of (SH^-)-met-hemoCD3, prepared by the addition of 10 eq. of NaSH to met-hemoCD3 (5.0×10^{-4} M), was diluted to 5.0×10^{-6} M in air-saturated phosphate-buffered solution (pH 6), λ_{max} at 422 nm, attributable to O_2-hemoCD3, immediately appeared (Fig. 2), suggesting that (SH^-)-met-

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hemoCD3 was converted to O_2-hemoCD3 by the excess O_2 in the medium.

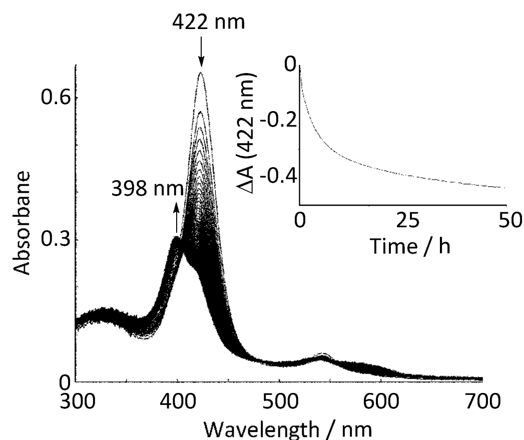
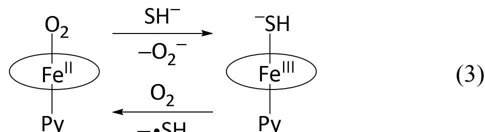


Fig. 2 Progressive absorption spectral changes of O_2-hemoCD3 (5×10^{-6} M) in the presence of 10 eq. NaSH in air-saturated phosphate buffer at pH 6.0 and 25°C.

Subsequently, a gradual shift of λ_{max} at 398-422 nm, which was ascribed to autoxidation of O_2-hemoCD3 to met-hemoCD3, was observed. The absorption spectrum of the final product showed that the autoxidation was not quantitative, and decomposition of Fe-porphyrin occurred (Fig. 2). The half-life ($t_{1/2}$) of O_2-hemoCD3 (autoxidation to met-hemoCD3) in the absence of NaSH at pH 7 is 51 h. However, in the presence of excess NaSH (10 eq.) at pH 6, autoxidation was accelerated ($t_{1/2}$ = 10 h), along with partial decomposition of Fe-porphyrin. Because O_2-hemoCD3 reacts with SH^-, reversible ligand exchange between SH^- and O_2 (O_2^-) generates •SH radicals and reactive oxygen species. Subsequently, these species may react with Fe-porphyrin and accelerate autoxidation [eqn. (3)]:



(3)

In summary, we found that hemoCD3 reacts with H_2S. Met-hemoCD3 yields a stable (SH^-)-met-hemoCD3 complex in oxygen-free aqueous solution. An Fe(III)-(SH^-) complex is generated from Fe(II)-O_2 as a result of ligand exchange between O_2^- and SH^-. In the presence of excess O_2, homolysis of the Fe(III)-S bond occurs and the Fe(II)-O_2 adduct is generated, along with partial decomposition of Fe-porphyrin. These findings imply that Hb catalytically mediates one-electron transfer from SH^- to O_2 and degrades H_2S *in vivo*. To the best of our knowledge, this is the first study to demonstrate the reversible reaction between SH^- and the O_2 adduct of the synthetic haeme model in aqueous solution.

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