# Synthetic Heme Thiolate Complexes as Precise Model of Cytochrome P450

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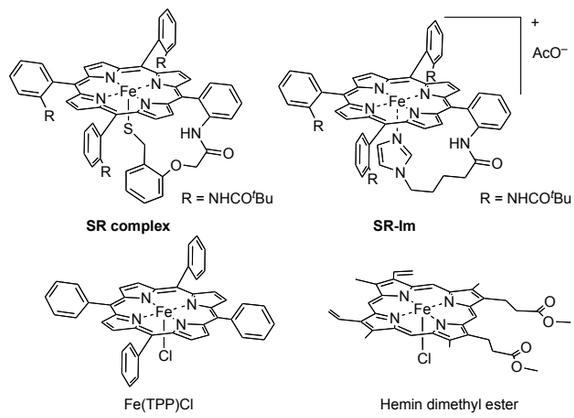
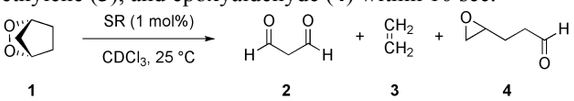
Cytochrome P450 plays a central role in drug metabolism and steroid biosynthesis. Among heme enzymes, cytochrome P450 and NO synthase (NOS) have strong oxidizing ability and unusual structure, in that their heme iron has thiolate coordination.

We previously succeeded in synthesizing the first synthetic heme thiolate (**SR** complex) which retains thiolate coordination during catalytic oxidation.[1)]




We report here the synthetic heme-thiolate complex-catalyzed isomerization of an endoperoxide (EP) and the large enhancing effect of the thiolate ligand on the reaction. Prostaglandin $H_2$ ($PGH_2$) isomerase is a cytochrome P450-type endoperxide-isomerizing enzyme.[2)] It would be of interest to know why nature has selected the heme-thiolate structure for the isomerization of EP, among a wide repertoire of metal complexes. Reactions of **1**, which is a partial structure of $PGH_2$, with iron porphyrins were monitored by $^1$H NMR measurement of samples obtained. In the presence of Fe(TPP)Cl or hemin dimethyl ester, **1** was completely unchanged in $CDCl_3$ at 25 ˚C for 24 h. In contrast, **SR** showed extremely high catalytic activity for EP isomerization/breakdown under the same conditions. Most of **1** was converted into malondialdehyde (**2**), ethylene (**3**), and epoxyaldehyde (**4**) within 10 sec.[3)]




**1** **2** **3** **4**

**SR-Im** complex, which is a mono-imidazole-ligated iron porphyrin, showed much lower activity than the **SR**. The initial rate of isomerization/breakdown by **SR** was almost *1,000-fold* higher than that by **SR-Im**. Coordination of chloride anion had no positive effect on the isomerization. This is the first example that axial ligand effect of thiolate on EP isomerization was unambiguously evaluated. Interestingly, the same type of conversion of **1** into **2** and **3** occurs in the reactions of $PGH_2$ and thromboxane synthase, prostacyclin synthase and cytochrome $P450_{CAM}$. The redox potential ($Fe^{III}/(Fe^{IV}$ or the electronic isomer)) of **SR** was lower than those of the other complexes. This is also the first report of the redox potential ($Fe^{III}/Fe^{IV}$) of synthetic heme-thiolate. This ready accessibility of the high-valent iron form of heme thiolate is considered critical for O-O bond cleavage of EP, which has no active hydrogen.

Next, spin-trap experiments were carried out to examine the formation of radical species. Compound **1** was reacted with **SR** (2.5 mol%) for 0.5 sec at 25 ˚C in benzene and then 3,3,5,5-tetramethylpyrroline-*N*-oxide (TMPO) was added to the mixture. The ESR spectrum of the reaction mixture clearly showed a sextet signal assignable to an alkoxyl radical-derived product, since its hyperfine coupling constants ($a_N$: 1.32 mT, $a_{\beta H}$: 0.58 mT) were in good agreement with those of TMPO-*tert*-butoxyl radical adduct ($a_N$: 1.33 mT, $a_{\beta H}$: 0.58 mT). This is the first direct evidence for formation of an oxy radical intermediate in the isomerization of EP catalyzed by heme.

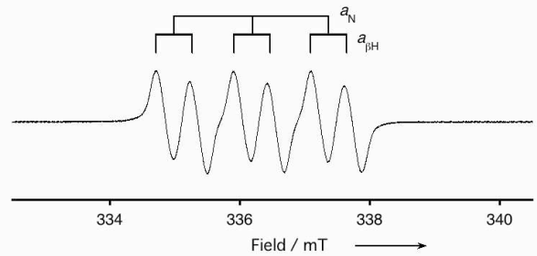



***Figure 1.*** ESR spectrum of radical adduct with TMPO. The reaction was carried out in benzene at 25 ºC. Substrate **1**: 6.0 mM, **SR**: 2.5 mol%, TMPO: 60 mM.

We propose that the reaction mechanism of isomerization of EP by heme-thiolate is as follows. Rapid homolytic cleavage of the O-O bond of EP coordinated to the iron atom occurs with the assistance of the potent electronic "push effect" of the thiolate ligand. The biradical then splits to give products **2** and **3**. As another pathway, oxy-radical coordinated to heme can rearrange to afford unsymmetrical **4**.

In summary, we have found an extremely strong enhancing effect of a thiolate axial ligand on the isomerization of EP catalyzed by heme in hydrophobic media. *This relative axial ligand effect is largest among heme-peroxide chemistry*. The thiolate ligand is suggested to play a critical role in cleavage of the O-O bond in the absence of the active hydrogen that hydroperoxides have. The present results provide strong evidence for the critical role of heme-thiolate structure for effective function of $PGH_2$ isomerase in a hydrophobic environment.

References

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