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# Lessons from Enzymes and Enzyme Models

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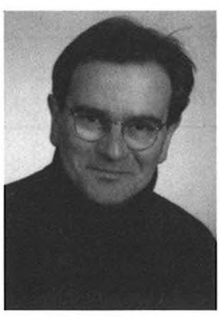
*Abstract.* Results from our laboratory are presented demonstrating the significance of synthetic active-site analogs of metalloproteins to accomplish catalytic enzyme-like reactions and to identify key intermediates of the reaction cycles.



*Wolf-Dietrich Woggon* was born in Berlin, did his undergraduate studies in geology and chemistry at the FU Berlin and then moved to the University of Zurich to obtain his Ph.D. in organic chemistry in 1975 under the supervision of *H. Schmid*. After a postdoctoral stay in the laboratory of *A. Battersby* in Cambridge, he returned to Zurich and completed his habilitation at the university in 1985. Thereafter, he established an independent research group and was appointed as a Professor of organic chemistry at the University of Basel in 1995.

## Introduction

Our research is concerned with enzymes that catalyze unusual reactions and involves the purification of unknown enzymes, the study of their mechanisms and

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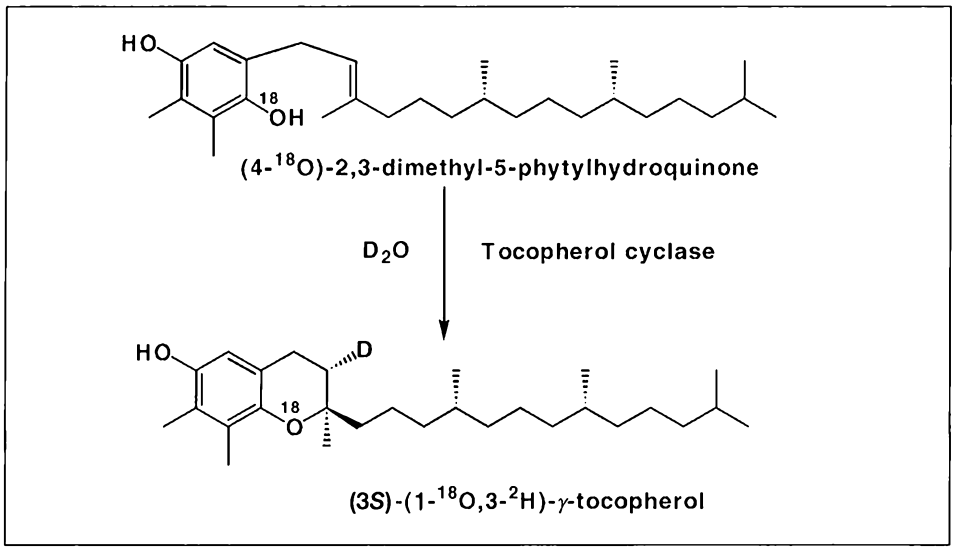
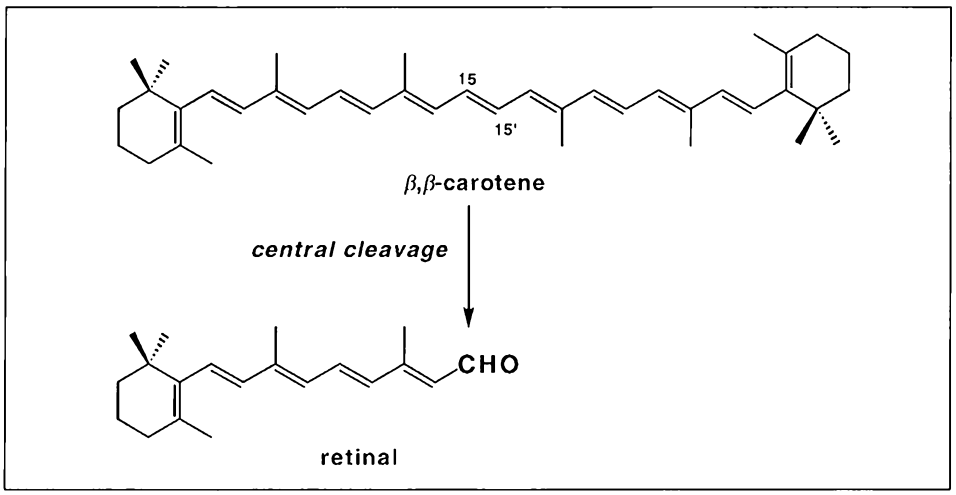
the preparation of synthetic enzyme models. The focus on analogs evolved because we believe that catalytic systems can display a reactivity comparable to enzymes, and that intermediates of the reaction cycles can be synthesized which have so far escaped detection. Provided these synthetic intermediates are structurally well-characterized and kinetically active, this may allow us to identify the corresponding intermediates in the protein. This research at the interface of chemistry and biochemistry provides information which often cannot be obtained from the protein itself and increases our understanding of how nature catalyzes sophisticated reactions.

Current research projects comprise the purification of two enzymes which are important for human nutrition, *tocopherol cyclase* (*Scheme 1*) and *β,β-carotene 15,15'-dioxygenase* (*Scheme 2*), and the synthesis of corresponding enzyme models. Further activities focus on the synthesis and catalytic efficiency of active-site analogues of metalloenzymes such as *cytochrome P450*, *chloroperoxidase (CPO)*, *vanadium chloroperoxidase*, and other *non-heme oxygenases*. Results from heme-thiolate projects are discussed below.

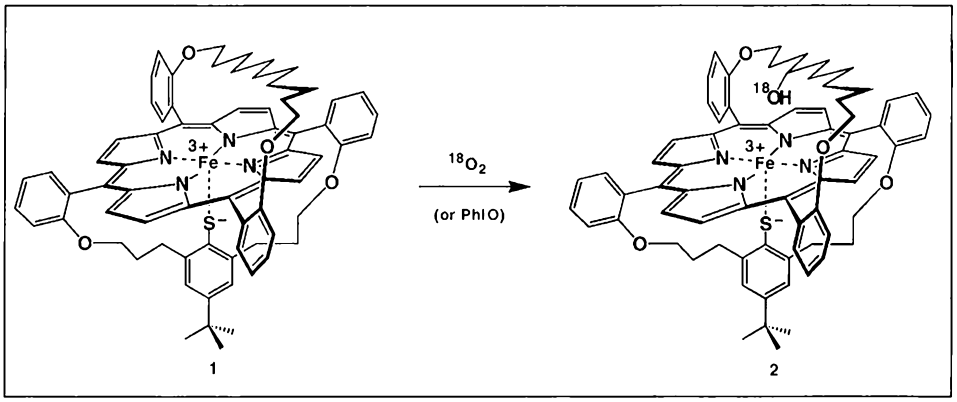
*Scheme 1*




*Scheme 2*




*Scheme 3*



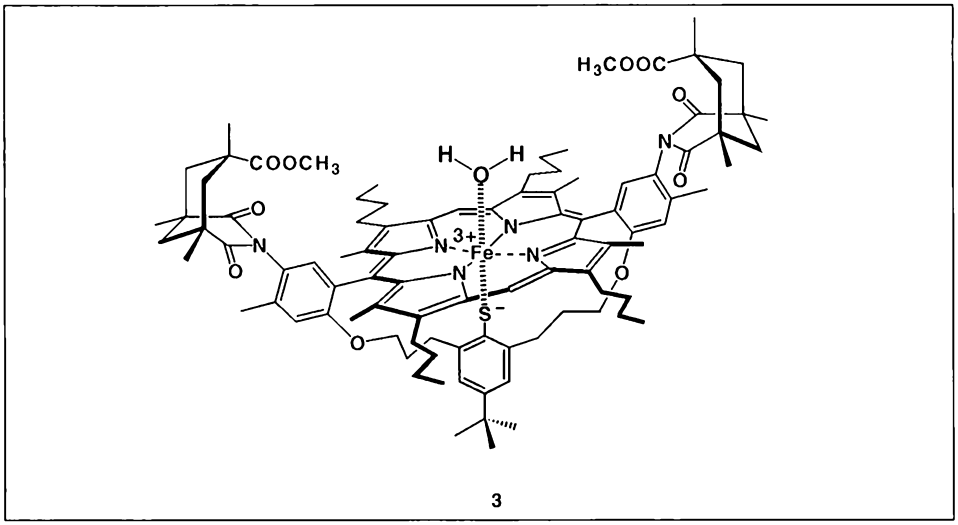

## Heme-Thiolate Proteins

The heme-thiolate proteins comprise a large number of different enzymes, such as chloroperoxidase (CPO) [1], cytochrome P450 [2], and nitric oxide synthase [3]. The inherent reactivity of these enzymes is attributed to an iron(III) protoporphyrin IX complex at the active site bound to the protein by hydrogen bonds to the two propionates of the heme. Most significantly, a thiolate ligand derived from cysteine in a highly conserved area located at the face of the porphyrin opposite to the substrate and oxygen binding site is coordinated to the iron centre. The thiolate ligand plays an important role in the reactivity of the prosthetic heme group [4] and controls the redox potential of these enzymes [5].

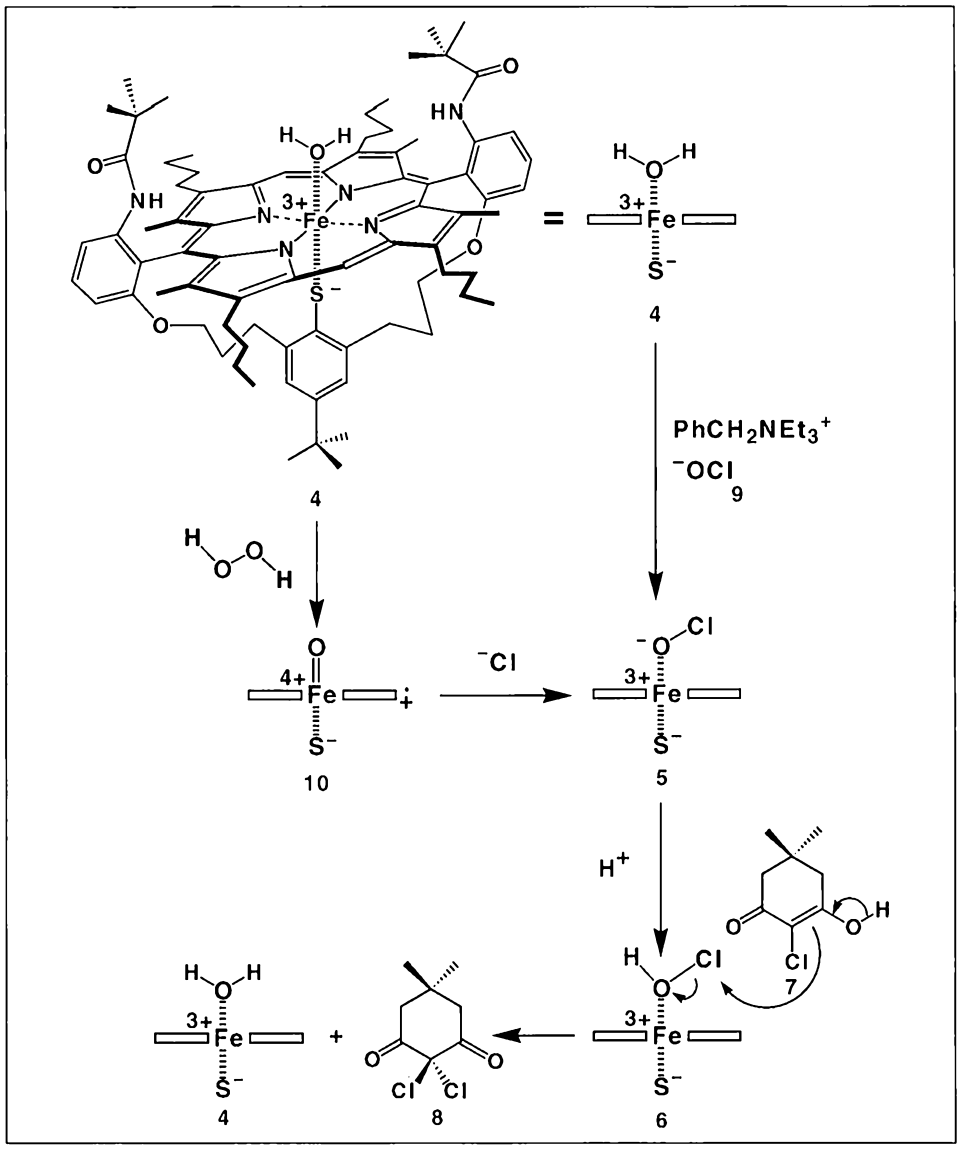
The ubiquitous *cytochrome P450* enzymes are important in the metabolism of endogenous compounds and xenobiotics [2][6]. Our knowledge of intermediates in the catalytic cycle relies on X-ray structures of different forms of cytochrome $P450_{cam}$ [7][8] and stems from investigating suitable model compounds [6].

From these studies, a generally accepted reaction scheme evolved; however, several aspects have recently been questioned. Contributions from our laboratory include the synthesis of the first model **1** [4] which cleaves molecular oxygen and intramolecularly hydroxylates non-activated C–H bonds (see **2** [9], *Scheme 3*), and the use of cytochrome $P450_{cath}$ from the plant *Catharanthus roseus* to demonstrate that allylic hydroxylation of geraniol proceeds with retention of configuration [10]. More recently, we have studied iron porphyrins with different thiolate ligands [11] and have shown that the Fe(II)/(III) redox potential is controlled by the thiolate coordination. This allowed an interpretation of the relatively positive redox potential of enzymes such as $P450_{cam}$. We have also synthesized **3** with an axially coordinated water molecule and substrate recognition sites [11] which turned out to be useful as an active-site analog of the resting state of $P450_{cam}$.

In contrast to the resting state of cytochrome $P450_{cam}$, which also has one water molecule coordinating to the iron and is low-spin [12], **3** is high-spin [13]. This surprising discrepancy indicates that the coordination of water to the active site of cytochrome P450 is not the single determining factor in establishing the low-spin character of the system. It seems likely, as suggested by INDO/ROHF and molecular-dynamics simulations of cytochrome $P450_{cam}$ [14], that the electrostatic potential of the protein plays a decisive role in stabilizing the low-spin rest state of the enzyme.




Scheme 4




*Chloroperoxidase* (CPO) is the most versatile of the heme thiolate enzymes, catalyzing the chlorination of activated C–H bonds employing $H_2O_2$ and $Cl^-$ at pH 2.7 and reactions reminiscent of peroxidases, catalase, and cytochrome P450. Despite numerous investigations of the enzyme [15], the identification of significant reactive intermediates remained elusive.

We recently prepared **4**, a new model of CPO, and showed that it forms stable $^-$OCl and HOCl adducts, **5** and **6**, under strictly defined conditions [16]; only **6** is catalytically active in chlorinating **7**, the substrate in the standard assay of CPO (*Scheme 4*) [17]. If the proton in **6** is replaced by a *Lewis* acid, turnover numbers of up to 1600 can be achieved. It is important to note that **5**, and subsequently **6**, were produced from **4** by two independent reaction pathways using benzyl(triethyl)ammonium hypochlorite **9**, and $H_2O_2$ together with benzyl(triethyl)ammonium chloride. In particular, the latter, enzyme-like conditions, suggested that **5** and **6** are kinetically active analogs of intermediates in the catalytic cycle of the enzyme CPO.

Subsequently, the spectroscopic parameters of the active-site analogs **5** and **6** were employed to identify the previously elusive intermediates in the CPO reaction sequence [18]. These experiments established a plausible reaction mechanism of chloroperoxidase (*Scheme 5*) [18][19]. In contrast to earlier hypotheses which suggested a 'free halogenating species' released from CPO, our results clearly indicate that **6** is the catalytically active $Cl^+$ donor.

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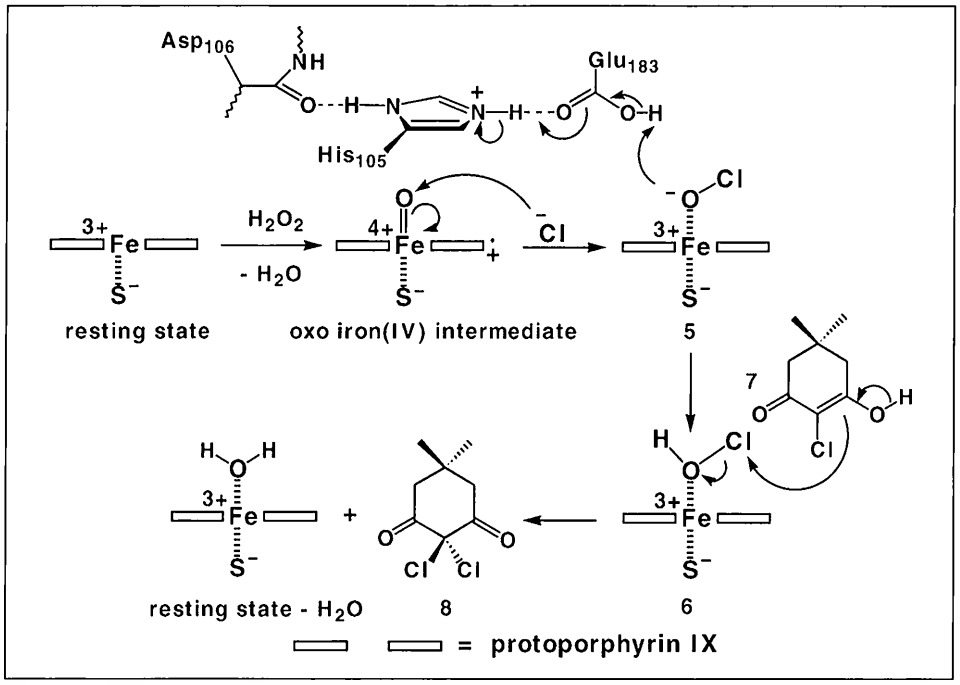
*Scheme 5*

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