Stereoselective Oxygenation

An Active-Site Model of Prostaglandin H Synthase: An Iron "Twin-Coronet" Porphyrin with an Aryloxyl Radical Overhang and Its Catalytic Oxygenation of 1,4-Diene**

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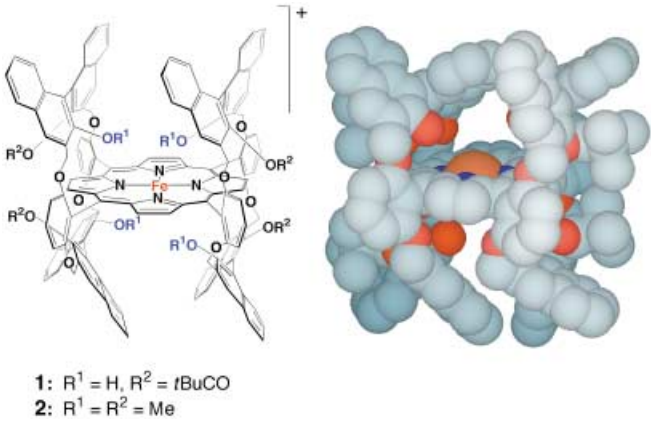
The tyrosyl radical acts as a mediator for electron-/proton-transfer reactions coupled to metal redox centers in various metalloenzymes in spite of its instability under aerobic conditions.[1] Among several metalloenzymes utilizing the tyrosyl radical, prostaglandin H synthases (PGHSs), which catalyze the addition of two equivalents of dioxygen to arachidonic acid (AA) in the formation of prostaglandin H_2 (PGH_2), contain a tyrosyl residue (Tyr385 for PGHS-1) located 10 Å from the heme unit in the peroxidase active site.[2] The essential steps of the reaction are 1) generation of the tyrosyl radical (Tyr-O•) by a rapid intramolecular electron transfer from tyrosine to an oxoiron(IV) porphyrin π cation radical and 2) regio- and stereoselective H• radical abstraction of the 13-*pro-S* hydrogen from AA and trapping of the resultant radical with O_2.[3] The intrinsic instability of aryloxyl radicals becomes an obstacle in aryloxyl-radical-mediated organic synthesis[4] in spite of its potential utility. Herein we report the formation of an aryloxyl radical close to the metal center of a binaphthol-bridged iron "twin-coronet" porphyrin (TCP), which is investigated as a PGHS model, and its catalytic and stereoselective hydroxylation of 1,4-diene under aerobic conditions. Ferric TCP **1**,[5] which has D_2 symmetry, has four naphthol groups, tightly bound between *meso*-phenyl groups, which form rigid, hydrophobic, chiral cavities on both faces of the porphyrin ring (Figure 1). In this complex, hydroxy groups are situated in the cavity and do not directly coordinate to the Fe ion.

The oxidation of the model complex **1** with *m*-chloroperbenzoic acid (*m*CPBA) results in the formation of a new species **1′** distinguishable in the UV/Vis spectrum (Figure 2) with a concomitant color change from brown to red-brown, which is distinct from the typical *m*CPBA-oxidized species, $[Fe^{IV}(=O)\mathbf{P}]^{+\bullet}$, of an iron porphyrin (**P** = porphyrin).[6] The oxidized species **1′** exhibits absorption bands at 365 and 478 nm as well as at 402 (Soret) and 540 nm (Q), which are characteristic of an iron(IV) porphyrin.[7] The bands at 365 and 478 nm are assigned to the corresponding naphthoxyl radical (Np-O•) based on comparison of the spectroscopic evidence with the reported spectra of 2-nahthoxyl radical:[8] the ESR spectrum of **1′** (Figure 3) is dominated by a signal at $g = 2.0046$ no signals arising from Fe^{III} centers are detected after complete conversion. In a frozen solution, **1′** gives a single ESR signal without any splitting, though its solution spectra show hyperfine couplings assigned to the naphthoxyl radical.[9] No signals arising from to Fe^{IV} centers could be detected, because this species is ESR silent. Quantifying the radical by double integration with 2,2,6,6-tetramethyl-1-piperidinyloxy (TMPO) as an external standard showed that **1** was converted into the corresponding Np-O• in 74% yield, which indicates efficient formation of **1′** within experimental error.



1: $R^1 = H$, $R^2 = t$BuCO
2: $R^1 = R^2 = Me$

Figure 1. Left: Iron "twin-coronet" porphyrin **1,** as a PGHS model, and a methyl derivative (**2**). Right: Computer-generated model of **1**. Hydrogen atoms are omitted for clarity.

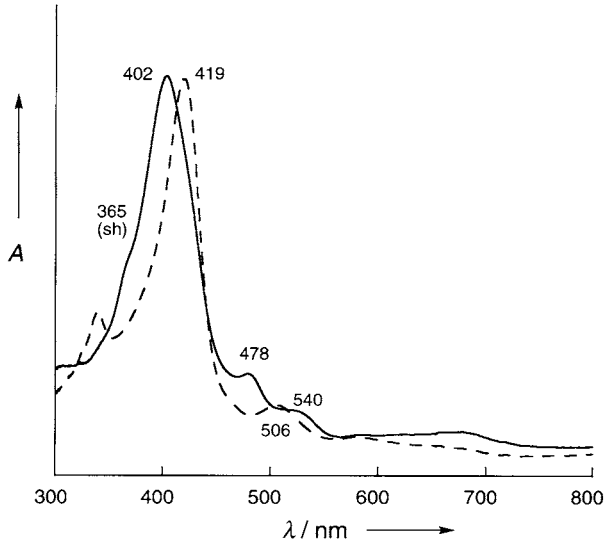


Figure 2. UV/Vis spectra of **1** (– – –, 1.66×10^{-5} M) and the oxidized species **1′** (——) with *m*CPBA (2.5 equiv) in CH_2Cl_2 at −40 °C.

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Supporting information for this article (spectral data on compounds **1** and **1′**) is available on the WWW under http://www.angewandte.org or from the author.

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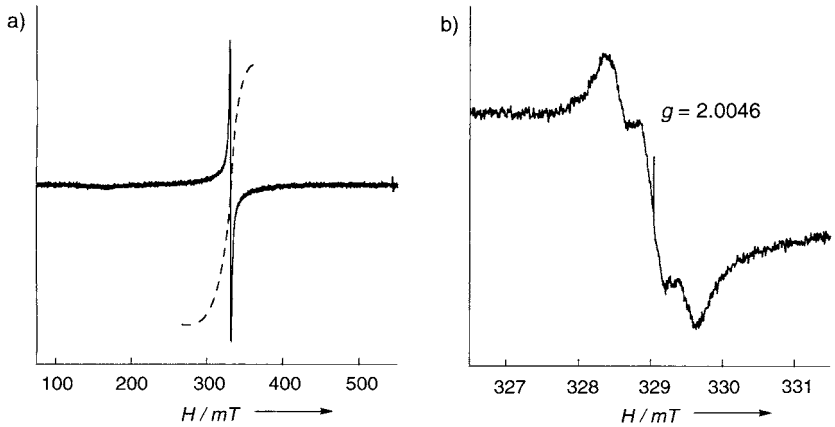

Figure 3. a) ESR spectrum of the oxidized species **1′** (——, 1.66×10^{-3} M) in CH_2Cl_2 at 77 K, and double integration of its signal (– – –). b) Its solution state ESR spectrum CH_2Cl_2 at 183 K. Magnification of $g=2$ region.

In resonance Raman spectroscopy of **1′**, selective excitation ($\lambda_{ex}=476.5$ nm) of the absorption band corresponding to the Np-O$^{\bullet}$ moiety indicates two scattered bands, 1579 and 1525 cm^{-1}, in the same region as the corresponding phenoxyl radicals (Figure 4).[10] Upon Soret-band excitation ($\lambda_{ex}=$ 406.7 nm), two porphyrin marker bands are observed at 1368 ($\tilde{\nu}_4$) and 1569 cm^{-1} ($\tilde{\nu}_2$). The latter is at higher wavenumber by 13 cm^{-1} than the corresponding band of the ferric species **1** (1556 cm^{-1}). These frequencies are very similar to those of the nonradical Fe^{IV} porphyrin species (1369 and 1565 cm^{-1}).[11] All the spectroscopic data support **1′** being an Fe^{IV} species, possibly Fe^{IV}=O, with a single naphthoxyl radical moiety. No scattered bands that could be assigned to $\tilde{\nu}$(Fe=O) are observed presumably because of this units instability and photolability, even at low laser power in a spinning cell (and in the presence of various axial bases).

Consequently, it is concluded that **1′** [Fe^{IV}(X)/Np-O$^{\bullet}$] (X = =O or axial ligands) is formed upon heterolysis of the perbenzoate adduct of the ferric porphyrin **1**, either through the compound I ([Fe^{IV}(=O)**P**]$^{+\bullet}$) and subsequent intramolecular electron transfer or through a concerted and direct pathway. The cyclic voltammogram of **1** in CH_2Cl_2 (0.1 M Bu_4NClO_4 as a supporting electrolyte) has an irreversible wave at $E_{pa}=+0.61$ V (vs. Ag/AgCl) and a quasi-reversible wave at $E_{1/2}=+0.77$ V. The former wave is assigned to the redox couple Np-OH/Np-O$^{\bullet}$[12] and the latter to Fe^{III}/Fe^{IV}.[13] Thus, the Fe^{IV}**P** has sufficient potential to oxidize the naphthol moiety.

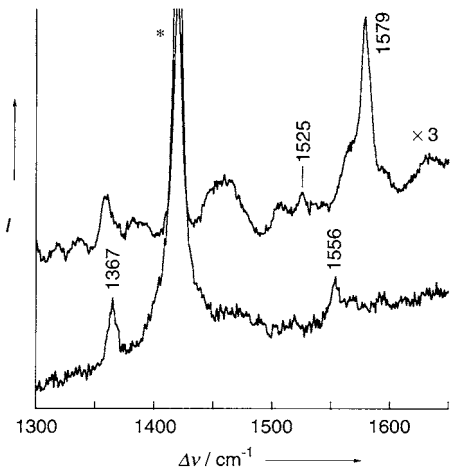

Figure 4. High-frequency region of resonance Raman spectra of **1** (lower line, 1.66×10^{-4} M) and the oxidized species **1′** (upper line). $\lambda_{ex}=476.5$ nm; *= solvent peak.

The titration of **1′** with *N*,*N*-dimethylaniline (DMA) as an efficient reducing agent[14] at −30 °C under N_2 indicates complete recovery of **1** upon addition of one molar equivalent of the substrate. There are clear isosbestic points in the UV/Vis spectra and a simultaneous decrease of the absorption bands corresponding to the Np-O$^{\bullet}$ and Fe^{IV} units (see Supporting Information). Evidence for this reduction process is also confirmed in the ESR spectra by the appearance of the signals assigned to Fe^{III} centers and the simultaneous decrease in the signals of the Np-O$^{\bullet}$ species upon addition of DMA. When 4-*cis*-7-*cis*-undeca-4,7-diene (**3**) is added instead of DMA under anaerobic conditions and the reaction is monitored by UV/Vis spectroscopy, the solution of **1′** also undergoes regeneration of **1**. Since, of the two redox active residues in **1′**, only the Np-O$^{\bullet}$ unit is considered to be reactive to the diene, these results suggest that both the Np-O$^{\bullet}$ and the Fe^{IV} units are involved in this reaction and intramolecular electron transfer from Np-OH to Fe^{IV}[15] occurs as the second step as a result of the higher oxidation potential of the $Fe^{III/IV}$ couple compared to the Np-OH/Np-O$^{\bullet}$ couple. Consequently, one **1′** molecule contains two oxidation equivalents which could react with two mole of the diene.

The radical intermediate **1′** was applied to the oxygenation of the 1,4-diene. This process is the fundamental reaction of PGH synthases. In the single-turnover reaction, addition of an excess amount of the diene **3** to the solution of **1′** generated by the *m*CPBA oxidation of **1** at −35 °C under O_2 atmosphere (1 atm) results in the formation of the corresponding dienyl hydroperoxide, which is stable at this temperature. After purging with N_2 and elevation to ambient temperature[16] (or treatment with Ph_3P), 5-*trans*-7-*cis*-undeca-5,7-diene-4-ol (**4**) was obtained in 156% yield, based on the amount of the iron TCP **1**, in good regio- and stereoselectivities (5*E*,7*Z*:5*E*,7*E* ≈ 5:1) as confirmed by GC-MS/^{1}H NMR spectroscopic analyses and by comparison with authentic samples. Further, this oxygenation is a catalytic reaction: **1**, diene (**3**, 30 molar equivalents relative to **1**), and *m*CPBA (12.5 molar equivalents relative to **1**) at −35 °C under O_2 give the dienyl alcohol **4** in 132% yield, based on the amount of *m*CPBA (catalyst turnover number (TON) = 16.5).

To rule out the possibility of oxidation side reaction(s) involving species other than **1′**, the reaction of the diene with several oxoiron species was investigated. When the [Fe^{IV}(=O)**P**]$^{+\bullet}$, formed by reaction of octamethyl TCP **2** with *m*CPBA, reacted with the diene **3**, only a trace amount

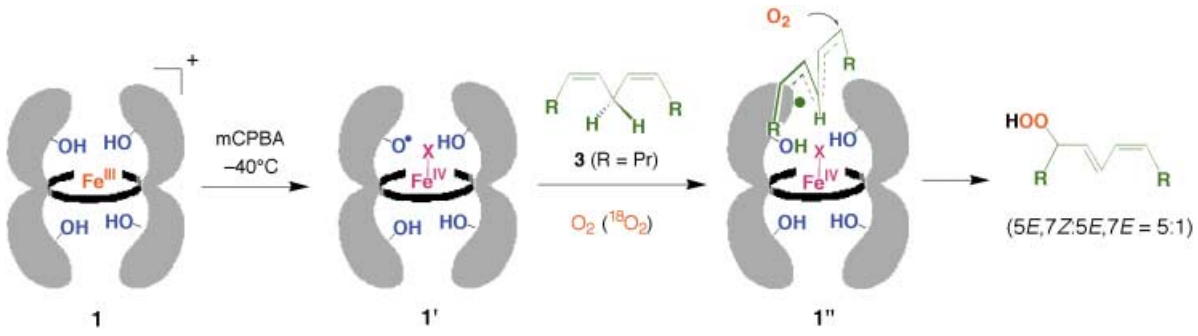

Figure 5. Formation of oxidized species **1**′ and regio- and stereoselective oxygenation of (4*Z*,7*Z*)-undeca-4,7-diene (**3**).

(< 5 %) of the corresponding monoepoxide was observed at −35 °C.[17] A separately prepared ferryl complex [Fe^{IV}(=O)**P**] (containing a different porphyrin ligand) does not show any reactivity toward the diene.[18] Thus, the observed dienyl alcohol **4** is formed only in the reaction with **1**′. Other potential oxidants can be excluded from the present oxygenation reaction. This oxygenation reaction with **1**′ could proceed through regioselective H· abstraction at 6-CH_2 of the diene by the naphthoxyl radical followed by stereoselective O_2 addition to produce the corresponding 5-*trans*-7-*cis*-4-hydroperoxyundeca-5,7-diene (Figure 5).[19] Since the hydroperoxide reacts very slowly with **1** below −30 °C, a stoichiometric amount of *m*CPBA is required for this catalytic oxygenation. It is worth noting that the observation of high stereoselectivity suggests that the resultant dienyl radical, generated after the hydrogen-atom abstraction step, is quickly trapped with O_2 in the cavity before it can escape to the solvent medium. The free stereoisomerization of the resultant pentadienyl radical could be restricted in the cavity formed by rigid naphthalene groups.

This oxygenation reaction is tightly coupled with the formation of dienyl radicals, because by-products, such as epoxides, ketones, are not detected in the reaction mixture. The alcohol product is not obtained in the absence of either of the components. A labeling experiment with $^{18}O_2$ (^{18}O, 95 %), indicates quantitative incorporation of labeled oxygen in the alcohol. Both [$^{18}O_2$]*m*CPBA and $H_2{}^{18}O$, which can act as alternative oxygen sources, do not give the labeled alcohol. Moreover, after the reaction, the catalyst **1** is recovered without decomposition.

In conclusion, FeTCP is considered to be a good active-site model for PGHSs. Selective hydroperoxidation of a 1,4-diene by this catalyst is also considered to be an artificial lipoxygenase reaction,[20] which usually results in a complex mixture of products.[21] This is the first example of a model system utilizing the unstable Ar-O· radical as a mediator in catalytic oxygenation reactions and provides important insights into the catalytic oxygenation mechanism of PGHSs.

Experimental Section

Complexes **1**,[5] **2**,[22] and (4*Z*,7*Z*)-undeca-4,7-diene,[23] were prepared as previously reported.

*m*CPBA oxidation of **1**: To a CH_2Cl_2 solution of **1** (1.66×10^{-5} M), *m*CPBA (2.5 molar equiv) was added at −40 °C in a 1 cm^3 cuvette (light-path length 2 mm) under N_2 atmosphere. The time-course of the UV/Vis spectral change was observed at 30 s intervals. ESR spectra were obtained at 77 and 183 K on a JES TE-300 spectrometer (JEOL) operating at 9.36 GHz equipped with a RMC CRYO SYSTEMS CT-470-ESR; microwave frequency: 100 kHz, modulation amplitude: 10 G, and microwave power: 1 mW. Resonance Raman spectra were obtained on a SpectraPro-300i spectrometer (Acton Research Co.) with a 2400-groove grating, a Beamlok 2060 Kr^+ laser and a Stabilite 2017 Ar^+ laser (Spectra Physics), a holographic supernoch filter (Kaiser Optical), and LN-1100PB CCD detector (Princeton Instruments) cooled with liquid N_2. Spectra were collected in spinning cells (2-cm diameter, 1500 rpm) at −80 °C at excitation wavelengths $\lambda_{ex} = 406.7$ or 476.5 nm (10 mW).

Catalytic oxygenation of (4*Z*,7*Z*)-undeca-4,7-diene (**3**): *m*CPBA (0.25 mL, 8.27×10^{-2} M in CH_2Cl_2) was slowly added to a stirred CH_2Cl_2 solution (10 mL) containing **1** (8.29×10^{-7} mole) and **3** (2.5×10^{-5} mole) at −35 °C under O_2 (or $^{18}O_2$) atmosphere (1 atm). After the reaction mixture was stirred for 30 min, portions of the *m*CPBA solution were added at 30-min intervals (0.25 mL × 4), after which the solution was stirred for a further 30 min, followed by substitution of the dissolved O_2 with N_2. The reaction mixture was allowed to stand to ambient temperature and was directly analyzed by a GC-MS (model JMS SUN200; JEOL), with tridecane as an internal standard. The resultant alcohols were isolated by flash column chromatography and their spectra were compared with authentic samples.

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[16] Upon being left to stand at room temperature, the hydroperoxide converts into the corresponding alcohol with a constant stereoisomeric ratio and without formation of any by-products by the ferric porphyrin catalyst **1**. This highly selective transformation of hydroperoxide/alcohol was confirmed by the separate reaction of the isolated hydroperoxide with **1**. The detailed mechanism will be discussed elsewhere.

[17] The reaction of the diene **3** with oxoiron(IV) porphyrin π cation radical **2′** at −35 °C also gave a trace amount (< 5 %) of the corresponding monoepoxide without any of the accompanying dienyl alcohols.

[18] *meso*-Tetramesitylporphinatoiron(II) was converted into the corresponding [Fe^{IV}(=O)**P**] species by the treatment with O_2 in toluene according to the reported method; A. L. Balch, *J. Am. Chem. Soc.* **1984**, *106*, 7779 – 7785. Addition of the diene **3** to this ferryl porphyrin solution at −35 °C resulted in the complete recovery of the diene. It is generally accepted that a ferryl complex does not catalyze H-abstraction or oxygen-atom transfer.

[19] Ordinary autoxidation of the diene in air gives the same hydroperoxide without any stereoselectivity (5-*trans*-7-*cis*:5-*trans*-7-*trans* = 1:1).

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