Highly Terminal-Selective Epoxidation of Linolenic Acid with an Amphiphilic Iron Porphyrin Catalyst Casted in Bilayer Membranes

Yoshinori Naruta,* Masa-oki Goto,† Toshifumi Tawara, and Fumito Tani
Institute for Fundamental Research of Organic Chemistry, Kyushu University, Higashi-ku, Fukuoka 812-8581
†*Department of Chemistry, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606-8501*

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An amphiphilic iron porphyrin having four hexadecanoic acid chains on each face of the porphyrin was prepared and the catalytic epoxidation of linolenic acid with the heme-casted bilayer membranes was achieved in high regioselectivity at the terminal double bond of the substrate. The orientation of the porphyrin ring in the membrane was determined to be parallel to the aqueous-bilayer interface by means of ESR.

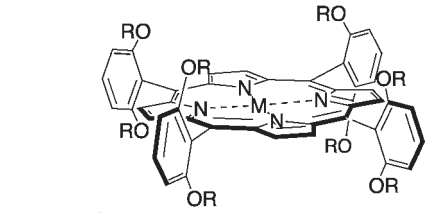
In organic synthesis, the discrimination of similar functional groups in one molecule both in electronical and sterical equivalence is difficult task. However, enzymes accomplish such selective molecular conversion with sophisticated molecular recognition system in them. For example, cytochrome P450BM-3 is capable to hydroxidize long normal saturated fatty acids (C12 – C18) at their terminal positions in good selectivity.[1] From X-ray crystallographic studies of substrate-bound[2a] and -unbound P450BM-3,[2b] the polar interaction between a Tyr group (Tyr 51) in the substrate access channel and the fatty acid carboxylate plays a major role to fix the terminal position of the substrate in close proximity to the reaction center. Though a functional model system of such substrate recognition was applied to the epoxidation of steroids and linoleic acid (two double bonds) with use of a steroid-linked porphyhrin in bilayer membranes, the discrimination of two double bonds in linoleic acid was incomplete.[3]

The key to accomplish good substrate recognition in an enzyme-mimic system is the fixation of the relative arrangement between the specific position of a substrate and the catalyst reaction center. The combination of a cationic quaternary ammonium amphiphile and an anionic carboxylate in a basic media could promote the fixation of their relative positions in close proximity by electrostatic interaction.

We report here the excellent regioselective epoxidation of linolenic acid by discrimination between its three cis double bonds with use of a new amphiphilic porphyrin catalyst in a bilayer membrane system.

We designed amphiphilic porphyrin **FeTP(C$_{16}$)P** as a catalyst, which has four fatty-acid chains on its both faces. In bilayer membranes, the eight polar anchors could stretch out the aliphatic chains on both of the bilayer surface. Consequently, the heme could be fixed in the middle of the bilayer keeping the heme plane parallel to the membrane surface, irrespective of the high flexibility of the long fatty acid chains. Electrostatic interaction is also expected between substrate linolenic acid and the cationic amphiphile (Figure 1).

The synthesis of the porphyrins was performed as follows: *meso*-Tetrakis(2,6-dihydroxyphenyl)porphyrin[4] was alkylated with methyl 16-iodohexadecanoate and K_2CO_3 under Ar to afford *meso*-tetrakis[2,6-di-(methoxycarbonylhexadecanyloxy)-phenyl]porphyrin **1**[5] in 17% yield. After iron insertion to **1**, **FeTP(C$_{16}$)P**[5] was obtained by hydrolysis of the resultant iron porphyrin under basic conditions (KOH-MeOH) and succeeding dialysis of the mixture with a cellulose membrane.



1, R = -(CH$_2$)$_{15}$CO$_2$Me, M = H$_2$
FeTP(C$_{16}$)P, R = -(CH$_2$)$_{15}$CO$_2$H, M = Fe(Cl)
CuTP(C$_{16}$)P, R = -(CH$_2$)$_{15}$CO$_2$H, M = Cu

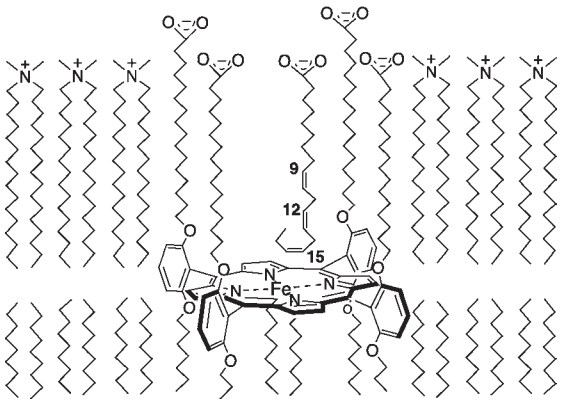


Figure 1. Schematic drawing of amphiphilic iron porphyrin **FeTP(C$_{16}$)P** and linolenic acid casted in DPDAB bilayer. DPDAB molecules around the substrate are omitted for clarity.

To inspect the applicability of the amphiphilic heme to the substrate recognition, epoxidation of linolenic acid having three consecutive cis double bonds was performed in a cationic bilayer membrane consisted of quaternary ammonium salt, dimethyldi-parmitylammonium bromide (DPDAB).[6] Reaction was performed with use of PhIO as an oxidant at 34 °C slight above the T_c (28 °C) of the bilayer in order to keep the sufficient fluidity of the membrane. The regioselectivity of the terminal olefin ($\Delta^{15,16}$) in the epoxidation reached to 82% (Table 1). In comparison with the statistical distribution of the isomers in the homogeneous system,[6] the observed selectivity in bilayer is very high. The present results exceeded those of the preceding examples.[3] Furthermore, the total yields of the monoepoxide and recovered starting material reached 93% in the bilayer system. Such high material balance suggests the suppression of the product decomposition and formation of the di- and triepoxides.

Table 1. Comparison of catalytic epoxidation of linolenic acid under different conditions

Catalyst	Reaction conditions	Reaction temp./°C	Isomeric ratio of epoxides/%[b] *cis*-$\Delta^{9,10}$	*cis*-$\Delta^{12,13}$	*cis*-$\Delta^{15,16}$	Terminal selectivity[c]	Epoxide total yield/%	Recovery /%
FeTPPCl[a]	homogeneous, CH_2Cl_2	0	22	34	44	0.5	12	46
FeTP(C_{16})PCl	DPDAB, pH 9	34	7	11	82	4.6	26	67

[a]TPP: *meso*-tetrakisphenylporphyrin. [b]Stereochemistry of the epoxides was determined by the comparison of their authentic samples. [c]Terminal selectivity = $[\Delta^{15,16}]/[\Delta^{9,10}+\Delta^{12,13}]$.

In order to verify our hypothesis of the catalyst location in membrane, we determined the orientation of the heme in the lipid bilayer by ESR. A Cu porphyrin is a good probe for the inspection of its orientation in the bilayer membrane, because of the clear differences between the isotropic and anisotropic spectra. **CuTP(C_{16})P**,[5] prepared in the similar way as the iron derivative, was incorporated into DPDAB bilayer and was deposited on Myler films.[7] The ESR of the film exhibited signals characteristic of a square planer complex of Cu(II) ion ($I = 3/2$) as shown in Figure 2. These anisotropic g ($g_\perp = 2.062$ and $g_{//} = 2.180$) and hyperfine splitting values ($A_{//} = 201\,cm^{-1}$) are resemble to the reported one of CuTPP (single crystal, $g_\perp = 2.045$, $g_{//} = 2.190$, $A_{//} = 209\,cm^{-1}$).[8] Thus, the simple saturated aliphatic acid chains linked to the heme are sufficient to fix the catalyst in the center of the bilayer with a desired orientation.

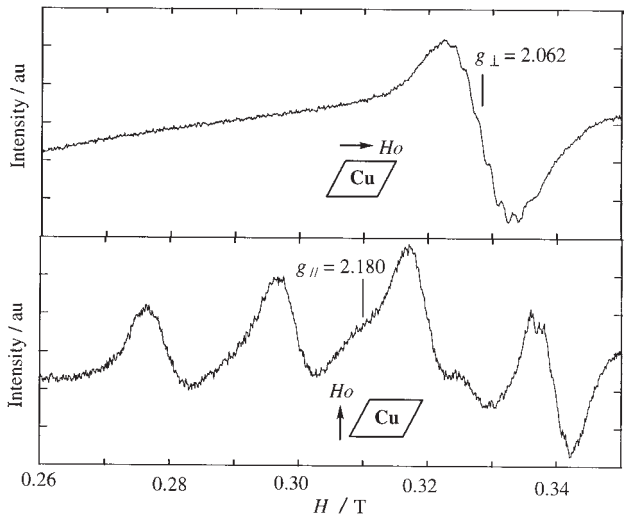


Figure 2. ESR spectra of **FeTP(C_{16})P** in an oriented bilayer assembly. (top) Magnetic field is parallel to the Myler film, $\theta = 0°$. Spectral conditions: microwave pw 1.0 mW, microwave freq. 9.447 GHz, modulation freq. 100 MHz, temp. 18 K. The angle θ is defined as the angle between the magnetic field and the normal to the plane of Myler strips.

In conclusion, we realized the regioselective epoxidation of linolenic acid and established that the electrostatic interaction between the heme, the substrate, and bilayer membrane worked as shown in Figure 1. When one applies this system to other substrates having different chain lengths, one could optimize the system just by choosing appropriate chain length for the catalyst and the amphiphile. In this way, the present system has enough flexibility to adopt substrates and can be regarded as the prototype of 'tailor-made' catalysts for terminal oxidation.

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This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 75th birthday.

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5 All new compounds gave satisfactorily analytical data. Selected data: **1**, mp 110–114 °C; ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 8H), 7.62 (t, 4H, $J = 8.3$ Hz), 6.94 (d, 8H, $J = 8.3$ Hz), 3.73 (t, 166H, $J = 6.8$ Hz), 3.65 (s, 24H), 2.28 (t, 16H, $J = 6.8$ Hz), −2.58 (s, 2H); IR (KBr) 2918, 2851, 1736, and 1464 cm^{-1}; MS (FAB, NBA) $m/z = 2890$; UV-vis (CH_2Cl_2); $\lambda = 420$, 515, 548, and 591 nm. **FeTP(C_{16})P**, ESI-MS $m/z = 2884$ $[M\text{-}2H\text{-}Cl]^-$; UV-vis (MeOH) $\lambda = 420$ and 548 nm. **CuTP(C_{16})P**, ESI-MS $m/z = 2892$ $[M\text{-}H]^-$; UV-vis (MeOH); $\lambda = 418$ and 543 nm.
6 In a homogeneous solution: To a CH_2Cl_2 solution of FeTPPCl (0.2 μmol) and linolenic acid (2 μmol), PhIO (2 μmol) was added and stirred for 3 h at 0 °C under N_2 atmosphere. After the evaporation of the solvent, the addition of stearic acid (2 μmol) as an internal standard for GC analysis, and succeeding treatment of the mixture with an ethereal solution of CH_2N_2, the resultant solution was analyzed by GC. In lipid bilayer: A $CHCl_3$ solution of linolenic acid (2 μmol), DPDAB (20 μmol), and **FeTP(C_{16})PCl** (0.2 μmol) in a round-bottom flask was evaporated to make a thin layer on the wall of the flask. Borate buffer (8 ml, pH 9.14, $Na_2B_4O_710H_2O$ 10 mmol in 1 kg water) was added into the flask, followed by N_2 substitution for 15 min. Ultrasonication of this mixture for 5 min at 50 °C (about 20 °C higher than T_c) with a probe-type sonicator (40–60 W output) under N_2 gave the transparent liquid of vesicles. A solution of PhIO (2 μmol) in MeOH/water (100 μl, 1 : 1 *v/v*) was added to the mixture at 34 °C under slow stirring of the mixture. After 3 h, the solvent was evaporated under reduced pressure followed by the same workup as that of the homogeneous system.
7 DPDAB vesicles containing 1 mol% **CuTP(C_{16})P** were prepared in borate buffer in the similar way as shown above. The resultant turbid vesicle suspension was spread on Mylar films. After drying in air, the homogeneously coated region of the film was cut into 3-mm strips and ten of these strips were put between two Teflon plates and all of them were banded together. This piece was placed in an ESR tube in N_2.
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