Syntheses and Catalytic Activities of New Cytochrome P-450 Model Compounds. Effect of Peptide Chains

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As cytochrome P-450 model compounds with structures similar to heme proteins, we designed and synthesized novel porphyrin iron (III) chloride complexes, **11** and **12**, which have three peptide chains (3PCs) and four peptide chains (4PCs), and evaluated their catalytic activities. The asymmetric porphyrin complexes are derived from etioporphyrin and peptide chains equivalent to proteins were provided by ring-opening polymerization of *N*-carboxy L-amino acid anhydride (L-Phe-NCA, γ-BLG-NCA, and N^{ϵ}-benzyloxycarbonyl-L-Lys-NCA) initiated by amino groups on meso positions. The new asymmetric porphyrin complex, **11** or **12**, was used for asymmetric epoxidations of styrene as is done by cytochrome P-450. The iron complex achieved the induction of asymmetry, although it has no special conformation, and gave *S*-styrene oxide in excess (ca. 60%ee). It was found, furthermore, that asymmetric induction was affected by the kind of amino acid residue, the number of peptide chains, and length of peptide chains.

Enzymes that make biological reactions proceed smoothly are important biological catalysts. The catalytic function, in general, is achieved by the cooperation of proteins and co-factors, such as coenzymes or metal ions. Nowadays a large number of enzymes are known to us: myoglobin and hemoglobin for oxygen storage and carriers, cytochrome P-450, tryptophanpyrrolase, and indolamine-2,3-dioxygenase as oxygenase, cytochrome c and b_5 as electron carriers, and peroxidase and catalase as peroxide decomposers, etc., are famous heme proteins. To discover the function of heme enzymes and heme proteins, porphyrin complexes have been widely investigated by many researchers. One of approaches is to use intact enzymes extracted from biological systems, and this method has been used for well-known heme proteins, such as hemoglobin. Results reported even in terms of hemoglobin, however, don't explain the relation between structure and function of heme enzymes completely, since it is discussed on the stage of phenomena and is not consistently understood on the level of molecules. Recently the approach making use of enzymatic model systems, on the other hand, has been increasingly active, because they can simplify the complexity and elaborateness of enzymes and step by step describe their functions. The information obtained from model systems cannot be used to explain the real function of an enzyme system itself, but the relation between structure and function of the model must contribute to understanding and discussion of the enzyme itself. From the latter standpoint, we have tried a molecular-level approach to the problem of sturcture and function of heme proteins.

The study on models of cytochrome P-450 as monooxygenases originated from the foremost study by Groves et al. in 1979.[1] They discovered that (tetraphenylporphyrinato)iron(III) chloride ([Fe(tpp)-Cl]) catalyzed the epoxidation of olefins in the presence of iodosylbenzene as the oxygen donor. The work has stimulated the use of synthetic porphyrins for modeling the oxygen-transfer reaction of cytochrome P-450. Furthermore, Groves et al. first synthesized porphyrin complexes having asymmetric space in 1983 and used them for asymmetric epoxidation.[2] Porphyrinatoiron-(III) chloride having amino acid such as L-phenylalanine as optically active substituents also was synthesized by Mansuy et al.[3] This metalloporphyrin was cleared to transfer oxygen catalytically and asymmetrically and afford optically active epoxides from prochiral styrenes. These P-450 models, in the vicinity of the porphyrin ring, contain stereoregulated space based on bulkier chiral substituents in the form of controlled conformation. Accordingly, it may be said that the P-450 models mentioned above are designed with a similar concept. In other words, the introduction of bulkier substituents having optical activity to the ortho position of the *meso*-phenyl groups, easily gives a stereoregulated chiral environment to a porphyrin ring together with the special conformation of the substituents.

Tetraphenylporphyrin derivatives as models of P-450 are more structurally similar to heme, with ease of synthesis and protection of the meso position having high reactivity. The P-450 models that have been proposed so far do not deviate from the above-mentioned concept and some of them have high enantioselectivity, but these model systems do not satisfactorily explain the relation of molecular structure with catalytic function of cytochrome P-450. Consequently, a unique model that can give some suggestion to the function of the enzyme from other aspects is desired.

We have synthesized cytochrome P-450 model compounds from the viewpoint of "biomimetic chemistry". As mentioned above, cytochrome P-450 is a heme protein. Heme proteins have conformations in which hemes are present in asymmetric space which proteins constitute, resulting in specific characteristics of this enzyme.

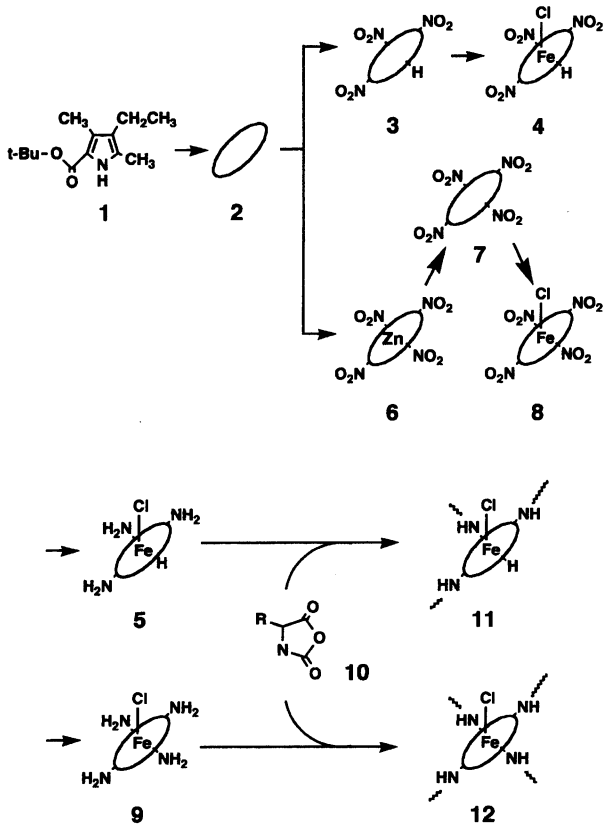
At present, however, there are no propositions for

models having segments mimicking proteins of this enzyme. New asymmetric porphyrin complexes having such segments were synthesized for this study. Furthermore, the complex was used for asymmetric epoxidation of styrene as is done by cytochrome P-450. The function of proteins in heme proteins will be discussed based on these results.

Experimental

Derivatives of Etioporphyrin. The compound was synthesized according to Scheme 1.

***t*-Butyl 4-Ethyl-3,5-dimethyl-2-pyrrolecarboxylate (1):** Distilled *t*-butyl acetoacetate (119.95 g) in glacial acetic acid (130 ml) was treated with aqueous sodium nitrite (75.05 g in 130 ml of H_2O) with stirring for 1 h at about 10 °C. The product was kept at 5 °C for an additional hour and at room temperature overnight. Then 3-ethyl-2,4-pentanedione (102.30 g) and acetic acid (150 ml) were added with stirring, followed by zinc dust (139.50 g) at such a rate as to keep the temperature at 80—85 °C. The reaction mixture was stirred for 30 min at this temperature and then heated to 100 °C for 2 h. The hot solution was poured, with stirring, into cold water, and the precipitated solid was separated and dissolved in chloroform. The chloroform solution was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The residue was recrystallized from methanol, and the ester **1** was obtained as colorless needles (32.9%, yield). IR (KBr) 3310, 1550 (=NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ=9.4—8.4 (1H, br, NH), 2.6—2.5 (2H, q, $C\underline{H}_2CH_3$), 2.3 (3H,s,5CH_3) 2.2 (3H, s, 3CH_3), 1.6 (9H, s, *t*-butyl), 1.3—1.0 (3H, t, $CH_2C\underline{H}_3$).



Scheme 1.

Found: C, 69.80; H, 9.74; N, 6.52%. Calcd for $C_{13}H_{21}NO_2$: C, 69.96; H, 9.42; N. 6.28%.

Etioporphyrin I (2): To *t*-butyl 4-ethyl-3,5-diethyl-2-pyrrolecarboxylate (**1**) (50.58 g) in glacial acetic acid (170 ml), bromine (35 ml) in glacial acetic acid (160 ml) was added dropwise for 3 h at room temperature. After vigorous reaction had subsided, the mixture was left overnight. The excessive bromine was removed by bubbling air into the system with an air-pump.

The resulting 5-bromo-5′-bromomethyl-3,4′-diethyl-4,3′-dimethyldipyrrin were filtered and washed with petroleum ether. The solid was dissolved in 100 ml of formic acid and refluxed for 3 h, and the solvent was evaporated off. The residue was dissolved in chloroform, washed with water, then saturated sodium hydrogen carbonate, and dried. The deep-purple crystals of etioporphyrin I (**2**) was obtained after recrystallization from dichloromethane-methanol (13.5% yield). ^1H NMR ($CDCl_3$) δ=10.1 (4H, s, CH), 4.3—3.9 (8H, q, $C\underline{H}_2CH_3$), 3.6 (12H, s, CH_3), 2.0—1.7 (12H, t, $CH_2C\underline{H}_3$), −4.2—−3.5 (2H, br, NH); UV-vis ($CHCl_3$) 398, 497, 532, 566, 619 nm.

Found: C, 80.48; H, 7.98; N, 11.49%. Calcd for $C_{32}H_{38}N_4$: C, 80.33; H, 7.95; N, 11.72%.

5,10,15-Trinitroetioporphyrin (3): Concentrated nitric acid (30 ml) was added to etioporphyrin I (**2**) (0.16 g) in concentrated sulfuric acid (30 ml) with cooling in an ice-bath. The mixture was stirred for 1 min, then immediately poured into water, and extracted with chloroform. The chloroform solution was washed with water, aqueous saturated sodium hydrogen carbonate solution, and water again, dried over Na_2SO_4, and evaporated. The product was chromatographed on silica gel (300 mesh) using chloroform/petroleum ether (6:4 v/v). The major fractions were collected, and evaporated. The residue was recrystallized from chloroform/methanol to give 5,10,15-trinitroetioporphyrin (**3**) (20.5%, yield). IR (KBr) 1535, 1363 (NO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ=9.9 (1H, s, CH), −3.9—−4.2 (2H, br, NH); UV-vis ($CHCl_3$) 385, 414, 508, 546, 584, 635 nm.

Found: C, 63.23; H, 6.25; N, 16.33%. Calcd for $C_{32}H_{35}N_7O_6$: C, 62.64; H, 5.71; N, 15.99%.

(5,10,15-Trinitroetioporphyrinato)iron(III) Chloride (4): To a solution of 5,10,15-trinitroetioporphyrin (**3**) (0.1 g) and 2,6-lutidine (0.9 ml) in tetrahydrofuran (30 ml), anhydrous iron (II) bromide was added under nitrogen. The reaction mixture was stirred overnight at room temperature and exposed to air for another hour. The solids were filtered off and washed with tetrahydrofuran. The solvent was removed by evaporation and the residue was dissolved in chloroform. The solution was shaken with 5% HCl until the visible spectrum was invariable and then dried over Na_2SO_4 and evaporated. The crude product was then chromatographed through silica gel (300 mesh) using chloroform/hexane (9:1 v/v) to afford (5,10,15-trinitroetioporphyrinato)iron(III) chloride (**4**) (51.2%, yield). UV-vis($CHCl_3$) 359, 563 nm.

(5,10,15-Triaminoetioporphyrinato)iron(III) Chloride (5): Palladium-on-carbon catalyst (6.2 mg, 10%) was added to (5,10,15-trinitroetioporphyrinato)iron(III) chloride (**4**) (61.8 mg) in N_2-purged tetrahydrofuran/methanol (25 ml, 8:2 v/v). The reaction mixture was stirred overnight at

50 °C under H_2 (50 atm). It was then filtered to remove the catalyst under nitrogen. After the solvent was evaporated the residue was recrystallized from pentane to give (5,10,15-triaminoetioporphyrinato)iron(III) complex (**5**) (97.0%, yield). IR (KBr) 3310, 3210, 1620 (NH_2) cm^{-1}; UV-vis ($CHCl_3$) 397, 530, 564 nm.

5,10,15,20-Tetranitroetioporphyrin (7): Zinc(II) nitrate hexahydrate (1 g) was added to etioporphyrin I (**2**) (0.134 g) in acetic anhydride (50 ml), and the mixture was stirred for 3 h at room temperature. It was then poured into water and extracted with chloroform. The chloroform solution was washed with water, saturated sodium hydrogen carbonate solution, and water again, dried over Na_2SO_4, and evaporated. Recrystallization from dichloromethane–methanol gave (5,10,15,20-tetranitroetioporphyrinato)zinc(II) (**6**) as deep green crystals. The (5,10,15,20-tetranitroetioporphyrinato)zinc(II) (**6**) (0.103 g) in dichloromethane (100 ml) was treated with perchloric acid (10 ml, 72%). After stirring at room temperature for half an hour, the solution was poured into water (50 ml). The organic layer was separated, and washed with water, saturated sodium hydrogen carbonate, and finally water. The solvent was removed by evaporation and the residue was recrystallized from dichloromethane–methanol to give 5,10,15,20-tetranitroetioporphyrin (**7**) (55.6%, yield). ^1H NMR ($CDCl_3$) $\delta = 3.3$—3.6 (8H, q, $C\underline{H}_2CH_3$), 1.4 (12H, s, CH_3), 2.0—1.7 (12H, t, $CH_2C\underline{H}_3$), −3.5—−3.0 (2H, br, NH); UV-vis ($CHCl_3$) 418, 521, 560, 602, 655 nm.

Found: C, 8.28; H, 4.98; N, 17.21%. Calcd for $C_{32}H_{34}N_8O_8$: C,58.36; H, 5.17; N, 17.02%.

(5,10,15,20-Tetranitroetioporphyrinato)iron(III) Chloride (8): To a solution of 5,10,15,20-tetranitroetioporphyrin (**7**) (0.2 g) in 40 ml of *N,N*-dimethylformamide (DMF), anhydrous iron(II) chloride was added under nitrogen. The reaction mixture was stirred for 20 min at 150 °C, in the dark and under nitrogen. Then the mixture was filtered and the solvent was removed. The residue was dissolved in chloroform, washed with water, dried over Na_2SO_4, and evaporated. The product was recrystallized from chloroform–methanol to give (5,10,15,20-tetranitroetioporphyrinato)iron(III) chloride (**8**) (80.0%, yield). UV-vis ($CHCl_3$) 370, 580 nm.

Found: C, 51.77; H, 4.26; N, 14.68%. Calcd for $C_{32}H_{32}N_8O_8FeCl$: C, 51.98; H, 4.28; N, 14.99%.

(5,10,15,20-Tetraaminoetioporphyrinato)iron(III) Chloride (9): Palladium-on-carbon catalyst (0.03 g, 10%) was suspended in 25 ml of dry methanol and sodium borohydride ($NaBH_4$) (0.08 g) was added. A slow stream of nitrogen was bubbled into the mixture, and (5,10,15,20-tetranitroetioporphyrinato)iron complex (**8**) (0.30 g) in 50 ml of dry tetrahydrofuran/methanol (8:2 v/v) was added over 5 min. After the reaction mixture was stirred for 10 min, it was filtered and thoroughly washed with N_2-purged water. The residue was dissolved in chloroform, washed with N_2-purged water again, and evaporated. Recrystallization from petroleum ether gave (5,10,15,20-tetraaminoetioporphyrinato)iron(III) chloride (**9**) (92.0%, yield). IR (KBr) 3330, 3230, 1620 (NH_2) cm^{-1}; UV-vis ($CHCl_3$) 405 nm.

Introduction of Peptide Chains. *N*-Carboxy L-Amino Acid Anhydride (NCA) (10): L-Amino acid (for example, 16.59 g of γ-benzyl L-glutamate) was added to 180 ml of distilled tetrahydrofuran and stirred at 40 °C. Trichloromethyl chloroformate (TCF) (12.68 ml) was added dropwise to the mixture at 40—50 °C for 3 h. γ-Benzyl L-glutamate was dissolved in the reaction mixture, as the reaction progressed. The solvent was removed by evaporation, and the residue was recrystallized from hexane to give the corresponding L-amino acid-NCA (for example, γ-BLG-NCA). γ-BLG-NCA: 51.3%, yield; IR (KBr) 1865, 1775 (acid anhydride, C=O) cm^{-1}; $[\alpha]_D^{23}$ −16.8° (*c* 3.15, AcOEt). L-Phe-NCA: 42.8%, yield; IR (KBr) 1845, 1775 (acid anhydride, C=O) cm^{-1}; $[\alpha]_D^{23}$ −24.5° (*c* 9.0, DMF); ε-Z-L-lysine-NCA (Z=benzyloxycarbonyl): 62.8%, yield; IR (KBr) 1850, 1775 (acid anhydride, C=O) cm^{-1}.

The Model Complex (11) (or 12)): A quantity of L-amino acid-NCA (for example, γ-BLG-NCA) was dissolved in 10—15 ml of distilled tetrahydrofuran and polymerized on **5** (or **9**) under nitrogen. The reaction was allowed to proceed for about 5 d. Disappearance of the absorption peak of acid anhydride (C=0) on infrared spectroscopy indicated completion of the reaction. The solvent was removed by evaporation and the residue was recrystallized from chloroform–methanol. Then this product was chromatographed on silica gel (300 mesh) to give **11** (or **12**). Average numbers of amino acids per a peptide chain were estimated from a calibration curve which was made with FT-IR.

Asymmetric Epoxidation. In a typical asymmetric oxidation reaction, 1 equiv of the metalloporphyrin catalyst, **11** or **12**, 100 equiv of iodosylbenzene, 500 equiv of styrene, and 5000 equiv of dry dichloromethane were used under anaerobic conditions. The reaction was initiated by the addition of iodosylbenzene, and the reaction mixture was stirred at −15±1 °C for 1 h. The product was isolated by silica-gel column chromatography using pentane/diethyl ether (85:15 v/v). The yields were measured by gas chromatography using polyethylene glycol (PEG)-20M Uniport HP 60/80. The ee was measured by ^1H NMR spectroscopy with tris[3-(trifluoromethylhydroxymethylene)-*d*-camphorato]europium(III) as a chiral shift reagent.

Results and Discussion

Preparation of Catalysts. Etioporphyrin was selected as a promising model to mimic cytochrome P-450. It is interesting that the active site of enzymes such as heme proteins, vitamin B_{12} and chlorophyll has the fundamental structure of etioporphyrin. Although many models of P-450 have been proposed, they often contain a tetraphenylporphyrin as the active center. This is due to the fact that the synthesis is easier and the complex is more stable because the meso positions with high reactivity are protected by phenyl groups. The porphyrin complexes, the meso positions of which are not protected, may be attacked by various reagents during a reaction, resulting in complete loss of the catalytic activity. On the contrary, we made good use of meso positions of porphyrin rings to introduce peptide chains as segments similar to the proteins of heme proteins.

Etioporphyrin I was synthesized from pyrrole derivative (*t*-butyl 4-ethyl-3,5-dimethyl-2-pyrrolecarboxylate) obtained by the method of Johnson et al.[4,5] The in-

troduction of three or four nitro groups were done by the use of different reagents; a nitric acid-sulfuric acid system for trinitroporphyrin,[6] and a zinc(II) nitrate hexahydrate–acetic anhydride one for tertranitroporphyrin.[7] The ^{1}H NMR analysis, in the former, is convenient for monitoring nitration of the meso positions. A singlet was observed at 9.9 ppm of one proton which remained in a meso position: the integral ratio between the singlet of this proton and the broad signal of NH at −3.9——4.2 ppm being 1:2. In the latter, on the other hand, there was no peak assigned to the proton on meso positions and only the broad signal of NH at −3.5——3.0 ppm was observed. According to ultraviolet-visible spectroscopy, the absorption peaks of Q band were shifted to longer wavelengths compared with those of etioporphyrin (for example, 497→508, 532→547, 566→584, and 619→635, on trinitroetioporphyrin), due to the electron-attracting nature of nitro groups. The inclination resembles the absorption intensity of the Q band of an etio-type changing and the position of absorption, at the same time, shifting to longer wavelengths, when electron-attracting groups (for example, aldehyde, ketone, and carboxylic acid groups) are directly attached to a porphyrin ring.

Metalation was done using anhydrous iron(II) chloride ($FeCl_2$) according to the method of Adler et al., in which the reaction solvent was *N*,*N*-dimethylformamide (DMF).[8] The metal-free porphyrin, tri- or tetranitroetioporphyrin, gave four absorption bands, extinction coefficients (ε) of which are about 10^4 at 480—650 nm, besides the Soret absorption band. However, the four bands that are attributed to **3** decreased to two bands upon the introduction of iron(III), while the peaks of **7**, to only one band (Fig. 1). Probably the superposition of absorption bands may occur due to the increase of symmetry in comparision with that of a freebase.

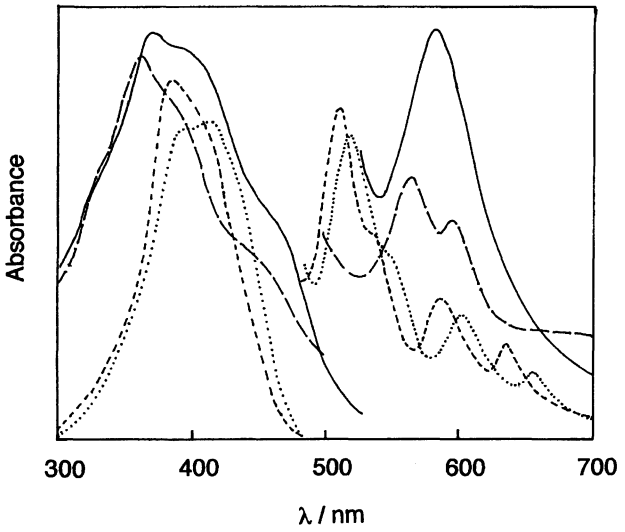


Fig. 1. Visible spectra of **3**, **4**, **7**, and **8** (in $CHCl_3$) : **3** (- - -), **4** (——), **7** (····), and **8** (—).

The reduction of nitro groups to amino groups of the complex was done by methods described in the experimental section. The amino groups obtained were very sensitive to oxygen, and were oxidized to nitro groups again or nitroso groups too immediately after short contact with oxygen (according to IR spectrum, the peaks of NO and NO_2 appeared at 1255 and 1530 cm^{-1}). Therefore, all of processes and treatments were done cautiously with exclusion of any trace of oxygen.

The resulting amino complex was allowed to react with γ-BLG-NCA or ε-Z-L-lysine-NCA by ring-opening polymerization to form an intended model complex. The reaction was completed in a few days. The data of infrared spectroscopy clearly indicated the formation of corresponding peptide chains: the disappearance of an absorption peak of acid anhydride (C=O) at about 1850 cm^{-1}, and the appearance of an absorption peak ascribable to amide bonds (CONH) at about 1670 cm^{-1}. Thus new cytochrome P-450 model complexes, **11** and **12**, were synthesized. The n value that expresses average numbers of amino acid residues per peptide chain was calculated from a calibration curve which was made with FT-IR.

Asymmetric Epoxidation of Styrene. A) Asymmetric Epoxidation Catalyzed by "3PCs" Type. A model complex having three peptide chains (3PCs) is shown in Fig. 2. As mentioned above, this is an original model containing segments similar to the protein of a heme protein. The model complex is characterized by the use of etioporphyrin I in place of tetraphenylporphyrin and the direct introduction of chiral substituents on the meso positions. This attempt makes the space of the active center wider as well as letting a substrate approach the chiral reaction field easily. Thus the environmental nature of the resulting model resembles that of heme proteins: the ease access of a substrate and a chirality provided by proteins.

Many kinds of chiral metalloporphyrins have been proposed for modeling an oxygen transfer reaction of cytochrome P-450. In the epoxidation of olefins in the presence of an oxygen donor such as iodosylbenzene, some of model systems have high stereoselectivities. The porphyrinatoiron complexes prepared by Naruta

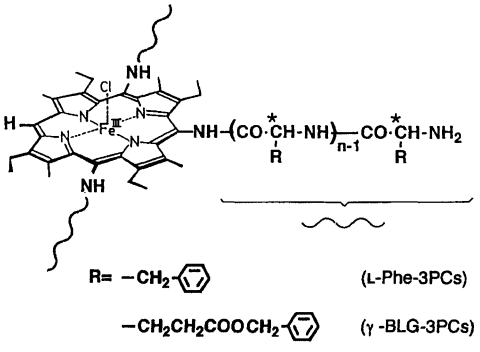


Fig. 2. "3PCs" type (=**11**).

et al., which have ligands of optically active bitetralin derivatives on both faces of the porphyrin ring, catalyzed epoxidations of styrene with 54% ee and 2-nitrosytene with the high ee of 89%.[9] Interestingly, charge-transfer (CT) interaction between a substrate and the chiral auxiliary moiety over the catalyst is estimated at the oxo transfer stage to result in the preferable fixation of the substrate and suitable recognition of the prochiral faces. Furthermore Groves et al. synthesized vaulted binaphtyl metalloporphyrins by an improved method of introduction of 1,1′-binaphthyl groups, which afford optically active epoxides from prochiral olefins.[10] For example, enantiomeric excess of 30% was obtained for styrene and high ee of 72%, for cis-β-methylstyrene. According to the authors, the stereo-electronic aspects of oxygen transfer from the metal center to an approaching substrate were taken into consideration for stereoselectivity on the basis of shapes of active site of the bridged porphyrin.

The model of "3PCs" type was applied to asymmetric epoxidations of styrene and the catalytic activity was evaluated. The results are shown in Table 1.

In model reactions, the complex having three peptide chains (n=4.5) of L-phenylalanine (L-Phe) as the amino acid residue catalyzed the model reaction, for example, to give enantiomeric excess as high as 60.6% and chemical yield of 56.0%. This result means that the ee may increase even if the space around an active center becomes wider, and also suggests the possibility of a model that can embody both specificity and high reaction rate of enzymes.

These model systems, which were proposed by Naruta et al. and Groves et al., are considered to induce high stereoselectivities by regulating reaction fields by ligands stereochemically. The fixed conformation in the vicinity of an active site is advantageous to fixation of substrate in a form convenient for recognizing the enantio-face of a substrate. On the contrary, the development of model complexes having no fixed conformation is very interesting in terms of observing asymmetric induction from a different standpoint. The complex having peptide chains proposed by us just corresponds to such a model.

Table 1 shows also that the asymmetric epoxidation was much affected by varieties of amino acid residues; when L-Phe was replaced by γ-benzyl-L-glutamate (γ-BLG), the chemcial yields of styrene oxide increased, for example, from 45.2 to 60.3%. However, the ee decreased greatly from 60.6 to 11.2%. This facts implies that the peptide chains of γ-BLG exist more freely than those of L-Phe in the reaction medium.

B) Asymmetric Epoxidation Catalyzed by "4PCs" Type. According to these results, the models of "4PCs" (four peptide chains) type were designed for the purpose of drawing out the role of PCs in more detail (Fig. 3). The use of such model complexes having γ-BLG units gave results as presented in Table 2. In model reactions mentioned before, the complex having three PCs of γ-BLG gave styrene oxide with rather low ee in spite of acceleration of reactions (for example, ee 11.2% at a yield of 60.3%). The model of four PCs, however, increased the ee considerably without much decrease in chemical yields (for example, ee 53.8% at a yield of 52.6%). This means that four PCs are in better interaction with the substrate, and also induce asymmetry without any fixed conformation. In addition, this result satisfies the original purpose that has been intended to draw out the role of PCs.

Data in Table 2 show that the length of a peptide chain also affects asymmetric induction dramatically: the ee increased from 16.9 to 53.8% in spite of similar chemical yields when average numbers of γ-BLG in a peptide chain increased from 2.0 to 4.4. This suggests that, even if chains are longer, the active site of complex opens spatially as in the shorter chains, but

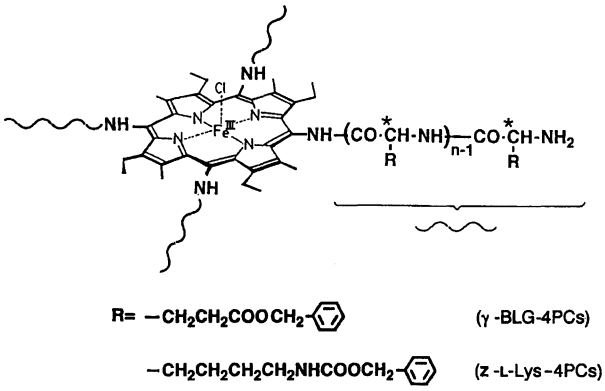


Fig. 3. "4PCs" type (=**12**).

Table 1. Epoxidation of Styrene Catalyzed by Porphyrinatoiron(III) Chloride with Three Peptide Chains

Catalysts	n[a]	Yield[b] (%)	Confign.	ee (%)
L-Phe-3PCs	3.4	56.0	S	49.8
L-Phe-3PCs	4.5	45.2	S	60.6
γ-BLG-3PCs	3.0	69.7	S	9.31
γ-BLG-3PCs	4.6	60.3	S	11.2

a) Average ratio of L-Phe (or γ-BLG)/Peptide chain. b) Based on iodosylbenzene.

Table 2. Epoxidation of Styrene Catalyzed by Porphyrinatoiron(III) Chloride with γ-BLG Peptides

Catalysts	n[a]	Yield[b] (%)	Confign.	ee (%)
γ-BLG-4PCs	2.0	55.9	S	16.9
γ-BLG-4PCs	4.4	52.6	S	53.8
γ-BLG-4PCs	4.4	47.6	S	50.7
γ-BLG-4PCs	6.2	7.24	S	39.1

a) Average ratio of γ-BLG/Peptide chain. b) Based on iodosylbenzene.

Table 3. Epoxidation of Sytrene Catalyzed by Porphyrinatoiron(III) Chloride with Z-L-Lys Peptides

Catalysts	n[a)]	Yield[b)] (%)	Confign.	ee (%)
Z-L-Lys-4PCs	2.3	24.8	S	20.1
	4.5	23.4	S	41.8
	4.5	24.4	S	54.7
	5.8	15.6	S	13.6

a) Average ratio of Z-L-Lys/Peptide chain. b) Based on iodosylbenzene.

as soon as the substrate is taken in, the conformation of peptide chains was transformed into a stereochemically preferred one, resulting in rather highly asymmetric epoxidation. However, when the number of γ-BLG was 6.2, both chemical yields and ee decreased. Probably, this may be due to decrease in the degree of freedom ascribable to the formation of helices, and longer peptide chains may cover porphyrin ring to shield the active center.

When amino acid residues were changed from γ-BLG to ε-Z-L-lysine, Z-L-lys-4PCs also catalyzed asymmetric epoxidation of styrene (Table 3). Z-L-Lys-4PCs provided generally lower chemical yields than γ-BLG-4PCs. This clearly indicates that the bulkiness of peptide side chains has a strong influence on the enantiomeric excess. Furthermore, the length of a peptide chain greatly affected asymmetric induction. This coincides with behavior of γ-BLG-4PCs for model reactions, that is the ee increased from 20.1 to 54.7% in spite of similar chemical yields when the number of ε-Z-L-lysine in a peptide chain was increased from 2.3 to 4.5.

The model complexes proposed by this research can be said to resemble cytochrome P-450 in many aspects, such as structure of etioporphyrin I, direct use of the meso position, combination of peptide chains with a porphyrin ring, and action of asymmetric epoxidation. These results are very interesting for understanding the role of proteins in the vicinity of an active center.

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