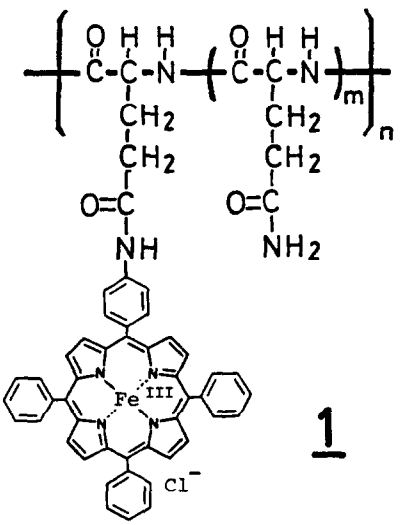
Tetrahedron Letters,Vol.26,No.45,pp 5555-5558,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

SYNTHESIS AND CYTOCHROME P-450-LIKE REACTIVITY OF POLYPEPTIDE-BOUND PORPHINATOIRON(III)[1)]

Takashi Mori, Tomofumi Santa, Masaaki Hirobe*
Faculty of Pharmaceutical Sciences, University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113, Japan

Summary: Polypeptide-bound porphinatoiron(III) was synthesized. This polymer could catalyze the hydroxylation of aniline with H_2O_2 more effectively, and catalyze the monooxygenase-type oxidation of olefins more selectively in the porphinatoiron (III)-O_2-$NaBH_4$-Me_4NOH system[2)] than non-bound porphinatoiron.

Cytochrome P-450 plays an important role in metabolizing biomolecules and xenobiotics. This enzyme can catalyze the oxidation of various substrates by the introduction of one oxygen atom into a substrate from molecular oxygen. Recently many attempts have been made to reproduce the reactivity in chemical systems with a simple metalloporphyrin. The hemin of cytochrome P-450 is, however, fixed in the apoprotein and the reactivity is thought to be influenced by the protein around hemin. In the cytochrome P-450 model reactions little attention has yet been paid to these environmental effects.[3)] Therefore we synthesized porphinatoiron(III) covalently bound to polypeptide (1) and examined its reactivity as a cytochrome P-450 model. In such a model compound, the formation of μ-oxo dimer of porphinatoiron should be inhibited, and furthermore the polypeptide chain around hemin should modify the reactivity.



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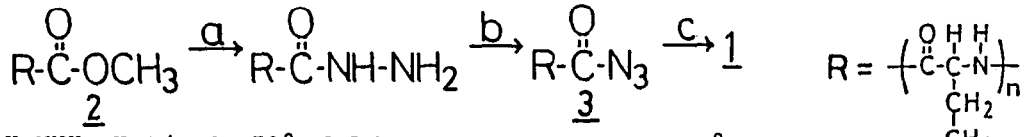
Polymer 1 was synthesized as follows. Films of poly-γ-methyl-L-glutamate (2)[4)] were converted into acyl azide films (3) according to Minamoto's methods[5)] with slight modifications. These acyl azide films were coupled with (5-p-aminophenyl-10,15,20-triphenylporphinato)iron(III) chloride (4)[6)] in abs. pyridine with heating. The heating and the use of pyridine as a solvent caused

the films to pulverize to afford the powdered polymer with a large surface area. Under these conditions acyl azide groups are known to be partially converted into isocyanate groups.[5] Porphyrins were thought to be linked to the polymer by either amide bonds as illustrated or ureido bonds (polymer-NHCONH-porphyrin). The unreacted acyl azide and isocyanate groups were converted into amide and ureido groups by aq. NH_3, then the polymer was washed with dil. HCl, water, and acetone until the washings were no longer colored to afford polymer 1-A (pale brown powder, mp>300°C; insoluble in water and all organic solvents tested). To examine the reproducibility of the method, the above procedures were repeated, and polymer 1-B was obtained. In the IR spectrum of polymer 1 the absorption bands of methyl ester (1730cm^{-1}), acyl azide (2180cm^{-1}) and isocyanate (2290cm^{-1}) were not observed, and amide bands (1655,1640cm^{-1}) were detected. The degree of incorporation of porphinatoiron into the polypeptide was determined by atomic absorption analysis. Porphinatoiron(III) 1.0 μmole was bound to 15.2 mg of polymer 1-A and 16.1 mg of 1-B. From this result the ratio of glutamine residues/porphinatoiron was estimated to be about 110. Polymer 1-A and 1-B contained almost the same amounts of porphinatoiron and gave the same IR spectrum, showing that the synthetic method is reproducible.



a; $H_2NNH_2 \cdot H_2O$/EtOH 70°C 2.5 hr, b; $NaNO_2$/0.2N HCl 0°C 30 min, c; 1) 4/ pyridine 20°C 24 hr → 50°C 48hr, 2) aq.NH_3, 3) 2N HCl.

In order to investigate the cytochrome P-450-like reactivities of polymer 1, we first examined the hydroxylation of aniline with H_2O_2 (Fig. 1).[7] When $FeCl_3$, $FeSO_4$ or non-bound porphinatoirons such as (5-p-acetamidophenyl-10,15,20-triphenylporphinato)iron(III) chloride ($FeM_{NHAc}PTPPCl$) and tetramesitylporphinatoiron(III) chloride (FeTMPCl) which is unable to form a μ-oxo dimer because of steric hindrance[8] were used, the yield of p-aminophenol based on aniline (based on porphyrin) did not exceed 0.2% (40%). On the other hand, when polymer 1-A or 1-B was used, it reached 4.9% (980%) or 4.1% (820%), respectively, at 3 min.[9] Polymers 1-A and 1-B had almost the same reactivity, so that the high reactivity of polymer 1 is reproducible. Under the same conditions, the consumption of aniline did not exceed 10% in the presence of non-bound porphyrin, while with polymer 1 it reached 35% (Fig. 2). Thus, the polypeptide-bound porphinatoiron (III) (1) was found to catalyze the oxidation of aniline far more effectively than non-bound iron compounds.

We have already reported the porphinatoiron(III)-O_2-$NaBH_4$-Me_4NOH system

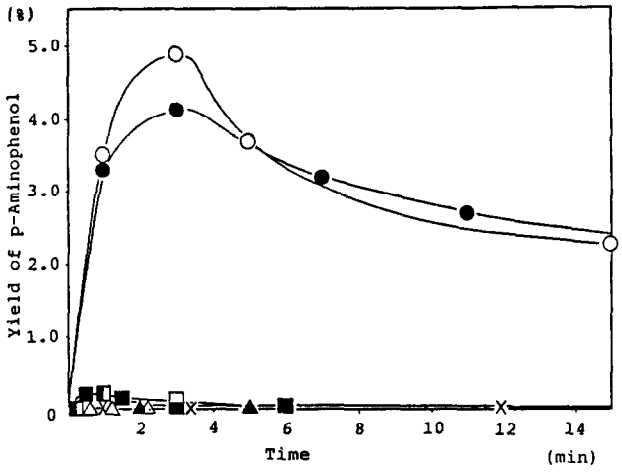


Fig.1. The p-Hydroxylation of Aniline. A typical procedure is described in note 7). -o- ; Polymer 1-A, -●- ; Polymer 1-B, -■- ; $FeM_{NHAc}PTPPCl$, -□- ; FeTMPCl, -▲- ; $FeCl_3$, -△- ; $FeSO_4$, -X- ; No catalyst.

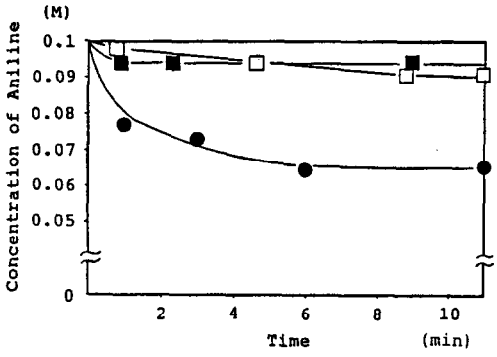


Fig.2. The Consumption of Aniline. A typical procedure is described in note 7). -●- ; Polymer 1-B, -■- ; $FeM_{NHAc}PTPPCl$, -□- ; FeTMPCl.

Table I. The Oxidation of Olefins in the Porphinatoiron-O_2-$NaBH_4$-Me_4NOH System.

Substrates	Products	Yields(%)[a] Polymer 1-A	Yields(%)[a] $FeM_{NHAc}PTPPCl$	Time(hr)
styrene	1-phenylethanol	97	100	0.5
cyclohexene	cyclohexanol	18	44	12
2-phenylpropane	2-phenyl-2-propanol	71 (5.9)[b]	41 (0.9)	1.0
	2,3-dimethyl-2,3-diphenylbutane	12	46	
1,1-diphenylethylene	1,1-diphenylethanol	71 (4.2)	40 (1.0)	14
	2,2,3,3-tetraphenylbutane	17	39	
trans-stilbene[c]	1,2-diphenylethanol	18 (0.75)	20 (0.37)	1.0
	benzyl alcohol	24	54	
cis-stilbene[c]	1,2-diphenylethanol	16 (0.84)	20 (0.37)	1.0
	benzyl alcohol	19	54	

A typical procedure was described in note 9). a, Based on olefins. b, The yield ratio of the hydroxylated product/another product. c, A mixture of benzene (1 ml) and methanol (1 ml) was used as a solvent.

as a cytochrome P-450 model.[2] In this system, tetraphenylporphinatoiron(III) activates molecular oxygen and catalyzes the hydroxylation, the coupling, and the cleavage of olefins. We next examined the oxidation of olefins in this system using polymer 1 (Table I).[10] $FeM_{NHAc}PTPPCl$ oxidized 2-phenylpropene into 2-phenyl-2-propanol (the hydroxylated product; yield, 41%) and 2,3-dimethyl-2,3-diphenylbutane (the coupled product; 46%), while with polymer 1 the coupling of the olefin was largely suppressed (12%) and the yield of 2-phenyl-2-propanol was increased (71%). Similar reactivity was seen when 1,1-diphenylethylene was used. $FeM_{NHAc}PTPPCl$ oxidized trans-stilbene into 1,2-diphenylethanol (the hydroxylated product; 20%) and benzyl alcohol (the cleaved product; 54%). The yield ratio of 1,2-diphenylethanol/ benzyl alcohol was 0.37. On the other hand, with polymer 1 the above ratio was increased to 0.75. The same reactivity was seen when cis-stilbene was used. From these results polymer 1 was found to catalyze the hydroxylation of olefins (the monooxygenase-type oxidation) more selectively than non-bound porphinatoiron.

As described above the polypeptide-bound porphinatoiron(III) (1) was found to have distinctive and interesting reactivities. Further studies on mechanism and various other reactivities of polymer 1 as a preferable cytochrome P-450 model compound are in progress.

Acknowledgement: We are thankful to Dr. Yoshiki Minamoto and Ajinomoto Co., Inc. for the supply of poly-γ-methyl-L-glutamate and far valuable advice.

REFERENCES AND NOTES

1) This forms part 9 of a series entitled "Chemical Studies on Drug Metabolism."
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3) Only a few studies have been reported on the reactivity of polymer-bound porphyrin as a cytochrome P-450 model compound; Alexander W. van der Made, Jan W. H. Smeets, Roeland J. M. Nolte and Wiendelt Drenth, J. Chem. Soc., Chem. Commun., 1204 (1983); M. Kuehn and P. Mohr, Die Pharmazie, 36, 383 (1981); M. Kuehn and J. Coupec, Z. Chem., 21, 231 (1981), and there has been no study on that of polypeptide-bound porphinatoiron.
4) Poly-γ-methyl-L-glutamate (AJICOAT A-2000. MW ca. 1.0×10^5) was supplied by Ajinomoto Co., Inc., Tokyo, Japan.
5) Y. Minamoto and Y. Yugari, Chem. Pharm. Bull., 28, 2052 (1980).
6) This porphyrin was synthesized by Tsuchida's methods. E. Tsuchida, E. Hasegawa and T. Kanayama, Macromolecules, 11 947 (1978). Iron was inserted by the use of $FeCl_2$ in refluxed DMF.
7) Typical procedure; A mixture of 30% H_2O_2 34 mg (H_2O_2 0.30 mmole) and methanol (2.0 ml) was added to a mixture of catalyst (Fe 1.5 μmole), aniline (0.30 mmole) and methanol (2.0 ml), then stirred at room temperature. p-Aminophenol was determined by HPLC. 30% H_2O_2 was purchased from Wako Pure Chemical Industries, Ltd.
8) Ru-Jen Cheng, Lechoslaw Latos-Grazynski and Alan L. Balch, Inorg. Chem., 21, 2412 (1982).
9) Other isomers, i.e., o- and m-aminophenol could not be detected under these conditions.
10) Typical procedure; Olefin (0.30 mmole), catalyst (Fe 1.5 μmole) and $NaBH_4$ (25 mg) were added to a mixture of methanol (2.0 ml) and 10% methanol solution of Me_4NOH (0.2 ml), then stirred vigorously. Products were determined by GLC and isolation.

(Received in Japan 29 August 1985)