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Pyridiniumporphyrin covalently bound to polystyrene: an efficient model of cytochrome P-450 reactivity

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Abstract

An easy and efficient method for preparation of *meso*-pyridinylporphyrins covalently bound by N-alkylation to polystyrene is provided. Catalytic efficiency of Mn^{3+} or Fe^{3+} metallocomplexes of pyridinium–porphyrin–polystyrene catalysts in styrene/*t*-butyl hydroperoxide or hydrogen peroxide/pyridine or *N*-methylimidazole/dichloromethane system is described. The effect of pyridiniumporphyrin isomerism on catalyst stability is studied.

Keywords: Cytochrome P-450; Epoxidation; Porphyrin

1. Introduction

Biomimetic models of cytochrome P-450 and other heme containing monooxygenases were found to be of great interest because of their use in elucidation of reactivity and mechanism of oxygen activation by monooxygenases [1]. During the last decade, numerous methods aimed to improve the catalytic stability of metalloporphyrin moieties have been utilized [1]. One of the ways to improve the stability of porphyrin catalyst is a method of site isolation of the catalyst units on the support, which may prevent porphyrin degradation by intermolecular reactions between catalyst sites [2].

Among the model systems of monooxygenase activity, the porphyrins bound to polymeric carriers have recently been found to be effective catalysts in many biomimetic reactions [3], chiefly hydroxylations [4] of saturated [5] or aromatic [6] substrates or epoxidations of unsaturated substrates [7,8].

Among the polymeric carriers, the modified polystyrenes seem to be advantageous for immobilization of porphyrin catalytic units. During the last few decades, such catalysts with an amino [9] or an ether [10] linkage between the porphyrin and carrier have been utilized. Thus Maruyama et al. [11] have used the chloromethylated polystyrene for quarternization of *meso*-pyridinylporphyrins to prepare quadricyclane isomerization catalyst. The disadvantage of this method is in the fact that chloromethyl groups of polystyrene are not very reactive towards pyridinylporphyrins. This is embodied in the fact that such porphyrins are for more than 95% bound to the carrier via one of the porphyrin pyridinyl substituents only, even if carriers with dense substitution (>50 mmol/g

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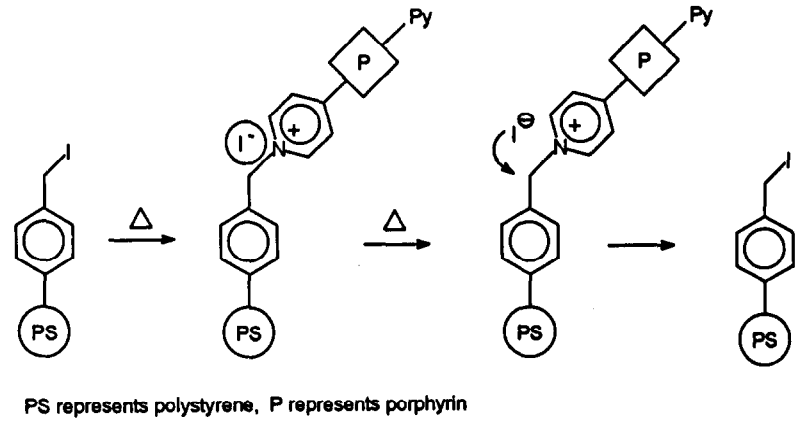


Fig. 1. Proposed mechanism for releasing of quarternized pyridiniumporphyrin unit from polystyrene.

of resin) and relative flexible polymer chains (1.5–2.5% of cross-linking) were used, as confirmed by IR spectroscopy.

We report here on an improved method for the preparation of pyridiniumporphyrin catalyst immobilized at polystyrene. Our synthetic approach consists of three steps: (i) conversion of the chloromethyl to more reactive iodomethyl groups (ii) quarternization of porphyrin pyridinyl substituents by iodomethyl groups of polystyrene and (iii) exchange of iodide counterion to perchlorate anion, since we detected that iodide counterion in further reactions (e.g., metallation of prepared catalyst) could attack the benzylic methylene group resulting in recovery of iodomethyl group and releasing of the porphyrin unit, according to the Fig. 1.

This improved method allows preparation of the catalyst particles with much higher density of substitution of such porphyrin catalytic subunits where the porphyrin moieties could be bound via two or more pyridinium substituents in porphyrin molecule (if present) and which could be, in the following step, converted to corresponding metallocomplex by transmetallation using acetylacetonate complex of the appropriate metal ion.

The catalysts were prepared from 5,15-bis-(2-pyridinyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphyrin, 5,15-bis(3-pyridinyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphyrin, and 5,15-bis(4-pyridinyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphyrin. The effect of connection position on catalyst stability has been investigated.

The catalytic properties were tested on model epoxidation reactions using styrene as a substrate and anhydrous *t*-butyl hydroperoxide (20% in dichloromethane) or anhydrous hydrogen peroxide (20% in *t*-butanol) [12] as a source of oxygen in the presence of heterocyclic base (pyridine or *N*-methylimidazole) as an axial ligand. The effect of pyridiniumporphyrin isomerism on catalyst stability was studied. Reactions were followed by GC.

2. Experimental

All compounds used in this study were of analytical grade purity. All solvents used were dry. Solvents used for washing the resins were of analytical grade purity exclusively.

Starting porphyrin compounds, i.e., 5,15-bis(2-pyridinyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphyrin, 5,15-bis(3-pyridinyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphyrin, 5,15-bis(4-pyridinyl)-2,8,12,18-tetraethyl-3,7,13,17-tetramethylporphyrin were prepared according to the literature [13].

Gas chromatography was performed on a Hewlett Packard 5890 instrument equipped with FID, Ultra 2 column, 50 m×0.22×0.32, GC–MS spectra were performed on a ZAB EQ instrument.

2.1. General procedure for the synthesis of pyridiniumporphyrin modified polystyrene

3 g of chloromethylated polystyrene (0.2 mmol Cl per gram of resin, 3% divinylbenzene) and dry sodium iodide (100 mg, 0.67 mmol) were suspended in absolute acetone (50 ml) and refluxed for 6 h. The resin was filtered off, washed three times with acetone (40–50°C), and dried in vacuum. The resin was then divided into three portions. Each portion was suspended in *N,N*-dimethylformamide (DMF) (30 ml) together with porphyrin derivative (2-, 3- or 4-pyridinyl isomer) (15 mg, 0.02 mmol) and the mixture was heated at 100°C for 10 h with bubbling of dry argon through a needle and occasional shaking. The resins were filtered off and washed with warm DMF and subsequently with chloroform in several portions, suspended in 1,4-dioxan (20 ml) together with magnesium perchlorate (100 mg, 0.45 mmol), and heated to 80°C for 3 h with argon bubbling. Catalysts were converted to their Fe^{3+} and Mn^{3+} metallocomplexes by refluxing in ethanol (30 ml) with appropriate acetylacetonate complex (0.28 mmol) for 24 h, after which the metallated resins were thoroughly washed with ethanol (40–50°C), 50% ethanol, water, diluted acetic acid, and ethanol and dried in vacuum. The content of metal ion in catalyst (regular content of catalytic sites) was determined by atomic absorption spectroscopy after mineralization in perchloric acid and shown to be 0.010 mmol/g of resin in case of the 2-isomer, 0.015 mmol/g of resin in case of the 3-isomer and 0.016 mmol/g of resin in case of the 4-isomer.

2.2. General procedure for epoxidation experiments

In a typical experiment, the catalyst (5 mg), styrene (50 mg, 0.52 mmol), *N*-methylimidazole (NMI) (10 mg, 0.12 mmol) or pyridine (10 mg, 0.13 mmol), and oxidizing agent either *t*-BuOOH solution in dichloromethane (2 ml, 4.44 mmol) or H_2O_2 solution in *t*-butanol (1 ml, 5.8 mmol) were suspended in dichloromethane (7 ml). Reaction was followed by GC by taking off the aliquots (0.5 ml), filtering and analysing. Reaction products were determined by comparation of retention times with those of authentic samples and GC–MS.

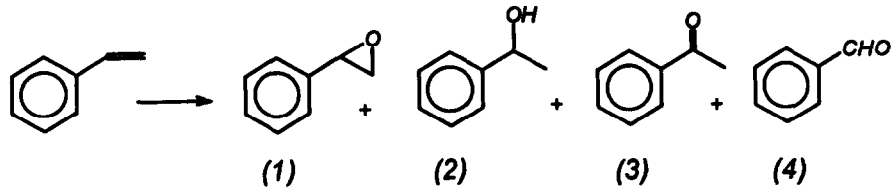
3. Results and conclusions

The epoxidation of styrene using *t*-butyl hydroperoxide afforded a complex mixture according to Scheme 1. Products **2–3** probably arose from styrene oxide (product **1**) which was found to be the main product ca 70%. 1-Phenylethanol (**2**), acetophenone (**3**) and benzaldehyde (**4**) (together 30%) which relative ratio to styrene oxide (**1**) (70%) has been found as 0.05:0.14:0.11 for Mn^{3+} and 0.05:0.15:0.10 for Fe^{3+} catalyst. Table 1 shows the conversion of substrate (%) after 12 h in a dependence on porphyrin isomer (polymer anchor position), central metal ion, and axial ligand used.

Under the use of hydrogen peroxide, the epoxidation mixture obtained was similar to that shown in the scheme above, with the following exceptions: The main product found was benzaldehyde (**4**) (80–90%); other products: styrene oxide (3–5%), acetophenone (4–10%) and combinations of other products which were not identified (3–5%). Table 2 shows conversion of substrate in % after 12 h as a function of porphyrin isomer (polymer anchor position), cation, and axial ligand used.

From our results we can draw the following conclusions:

First, the synthesis of this catalytically effective and stable polymer–pyridiniumporphyrin catalyst is extremely easy and efficient. This system, although it employs unhindered *meso*-diphenylporphyrin moieties, is superior in use in its stability against the formation of μ-oxo dimers, the active sites being held in defined distances controlled by the density of the substitution and, consequently, it provides high yields of products in model epoxidation reactions. The positive charges of pyridinium substitution probably play positive



Scheme 1. Epoxidation of styrene by *t*-butyl hydroperoxide or hydrogen peroxide.

role in increasing the catalyst stability by repulsion of catalyst subunits in case that they get closer to each other. Catalyst could not be leached out of the polymer, and it could be recycled. The position of porphyrin-carrier anchor (given by porphyrin pyridinyl substitution isomers) affects dramatically the whole catalytic activity. 2-Isomer, the most hindered one, exhibits lowest activity; on the other hand, the difference between 3- and 4-isomers seems to be quite small resulting in negligible differences in catalytic activity of both catalysts. The differences in catalytic activity of 2- vs. 3- and 4- catalyst isomers, are in our opinion, due to the fact that in case of the 2-isomer the pyridinyl nitrogens subjected to alkylation are extremely hindered and could not be twice alkylated by iodomethyl groups of polystyrene. This assumption was confirmed by IR spectra taken after the alkylation. While the 3- and 4- catalyst isomers exhibit ca. 45–75% and 50–75%, resp. of the product of bis-alkylation, the 2-isomer shows less then 15% of bis-alkylated product. This, together with fact that polymer carrier in case of 2-isomer probably limits the accessibility of central metal ion for the substrate and/or the axial ligand which results in probable collapse of the whole catalytic cycle [1] explains the lowest yields in model epoxidation reaction. The achieved conversions of the substrate prove that the catalyst is stable and gives results comparable to those obtained with hindered metalloporphyrin. These results represent a contribution to a general

Table 1
Epoxidation of styrene according to equation by porphyrin-modified polystyrenes using *t*-butyl hydroperoxide; % of conversion = sum of all products

Porphyrin isomer used:	Conversion of substrate (%)					
	2-Isomer		3-Isomer		4-Isomer	
Axial ligand:	Pyridine	NMI	Pyridine	NMI	Pyridine	NMI
Fe^{3+} catalyst	67	61	89	82	88	84
Mn^{3+} catalyst	75	70	93	88	94	91

Table 2
Epoxidation of styrene as in Scheme 1 by porphyrin-modified polystyrenes using hydrogen peroxide; % of conversion = sum of all products

Porphyrin isomer used:	Conversion of substrate (%)					
	2-Isomer		3-Isomer		4-Isomer	
Axial ligand:	Pyridine	NMI	Pyridine	NMI	Pyridine	NMI
Fe^{3+} catalyst	51	48	75	78	70	76
Mn^{3+} catalyst	66	67	83	82	87	83

assumption that the polymer backbone is capable to simulate apoprotein by creating appropriate conditions for the active sites and could play a significant role in improving the stability of simple unhindered diphenylporphyrin biomimetic models.

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