ELSEVIER

Journal of Molecular Catalysis A: Chemical 118 (1997) 63–68

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Porphyrins covalently bound to polystyrene II. an efficient model of monooxygenase reactivity

Pavel Anzenbacher Jr. [a,*], Vladimír Král [a], Karolina Jursíková [a], Jana Günterová [b], Alexander Kasal [b]

[a] *Institute of Chemical Technology, Dept. of Analytical Chemistry, Faculty of Chemical Engineering, Prague 6, Technická, 166 28, Prague, Czech Republic*

[b] *Institute of Organic Chemistry and Biochemistry, Czech Academy of Science, Flemingovo nám. 2, 166 10, Prague, Czech Republic*

Received 15 June 1996; accepted 28 August 1996

Abstract

A simple and efficient method for the preparation of metalloporphyrin catalyst bound to benzhydryl amine substituted polystyrene via amide bond was elaborated. Catalytic efficiency of the Fe^{3+} or Mn^{3+} metallocomplexes of these porphyrin–benzhydryl amido–polystyrene catalysts was studied at the epoxidation reactions in the systems styrene/iodosobenzene or *t*-butyl hydroperoxide/pyridine or imidazole (axial ligands). The rate of substrate conversion (turnover of the catalytic centre) as a function of the density of saturation of the benzhydryl amine substitution of the resin is discussed.

Keywords: Cytochrome P-450; Monooxygenases; Model; Epoxidation; Porphyrin

1. Introduction

Investigation of the monooxygenase enzyme systems has received an increased attention lately [1]. It is mainly due to the fact that these enzyme systems participate in the metabolism of many eobiotics and especially xenobiotics, and they also provide the possibility of being employed as alternative means of organic synthesis [2].

In connection with this, numerous porphyrin catalysts featuring enhanced stability towards oxidative degradation during catalytic process were prepared. One of the successful procedures proved to be the so-called 'method of site isolation' [3] of the catalytic centres on the resin which is capable of preventing degradative intermolecular reactions of the catalytic centres. As inert carriers of organic nature capable to provide suitable environment for the 'accommodation' of porphyrin catalytic centres, the derivatives of polystyrene are often utilized [4], as they are easily accessible and feature a variety of arrangements of polymeric chains and 'pore' shapes given by cross-linking. Although these methods afforded a number of catalysts with interesting properties, their preparation is not always straightforward [5].

In this paper we describe several variants of a simplified method utilizing for the creation of the connection between catalytic unit and poly-

* Corresponding author.

1381-1169/97/$17.00 Copyright © 1997 Elsevier Science B.V. All rights reserved.
PII S1381-1169(96)00376-7

mer the amide bond arising from the reaction of polystyrene, possessing benzhydryl amine (α-aminodiphenylmethane) substitution, with appropriate activated derivative (chloride, mixed anhydride, or active ester) of 5-(4-carboxyphenyl)-10,15,20-tri-(4-tolyl)porphyrin. The amide bond was chosen because of the ease of preparation of the respective amido derivatives, and also with respect to the assumption that it should be, as with the natural monooxygenase systems, sufficiently stable under conditions of the epoxidation reactions. This optimized method consists in (i) the transformation of carboxyporphyrin to the appropriate activated derivative and (ii) subsequent reaction of the activated derivative with aminopolystyrene. As the polystyrene resins, commercially available benzhydryl amine polystyrenes (0.2 mmol amino groups/g of resin; 3–4% cross-linking) were used. This method provides catalyst particles of a high density of saturation of the resin substitution. The unreacted amino groups were capped on reaction with pivaloyl chloride (affording pivaloyl amide terminations). Capping of the unreacted amino groups must be regarded as a very important step because, due to the relatively low cross-linking of the polymeric chain, the metalloporphyrin catalytic unit may come too close to the unreacted amino group of another chain of the polymer whereby interference with the catalytic process could take place. The formed amidoporphyrin catalyst is subsequently transformed to the appropriate metallocomplex by the metallation reaction with the Fe^{3+} or Mn^{3+} acetylacetonate complex.

It was found that the method of activation (character of the activated derivative) markedly influences the density of saturation of the polystyrene substitution with porphyrin derivative. The following activated derivatives of carboxyporphyrins were tested: acid chloride, adduct with dicyclohexylcarbodiimide, and mixed anhydride with ethyl formate.

The catalytic properties were tested on model epoxidation reactions using styrene as a substrate and iodosobenzene or *t*-butyl hydroperoxide (20% in dichloromethane) as a source of oxygen in presence of pyridine or imidazole as an axial ligand. Stability of the catalyst in various systems (catalyst/central ion/oxygen source/axial ligand) was studied as well as the dependence of the catalyst stability on the method of its preparation, i.e., especially on the density of saturation of the benzhydryl amine resin substitution.

2. Experimental

All compounds used in this study were of analytical grade purity. All solvents were made anhydrous using standard procedures.

The starting porphyrin derivative, 5-(4-carboxyphenyl)-10,15,20-tri-(4-tolyl)porphyrin was prepared according to the literature [6]. The benzhydryl amine resins were thoroughly washed with 1% triethylamine in chloroform before use. The elemental analysis of washed and dried resin showed a 0.33% content of N. The data of elemental analyses were found consistent with IR spectroscopy measurements. Gas chromatography of reaction mixtures was performed on a Hewlett Packard 5890 instrument equipped with FID and an Ultra-2 column, 50m × 0.22 × 0.32. GC–MS spectra were performed on a ZAB EQ instrument.

2.1. Procedures for the preparation of catalysts

2.1.1. Acid chloride procedure [7]

The porphyrin (0.50 g, 0.71 mmol) was suspended in thionyl chloride (5 ml) and the mixture was refluxed for 5 h, after which the excess of thionyl chloride was distilled off. The remaining solid was co-distilled with toluene (5 × 20 ml), the porphyrin residue (0.58 g) was dissolved in chloroform (10 ml) and added dropwise to the stirred, ice-cooled suspension of benzhydryl amine resin (5 g) in chloroform (30 ml)–triethylamine (5 ml). The suspension was stirred thoroughly in an ice bath for 30 min, after which the temperature was allowed to rise

to 20°C. After an additional 2 h of stirring the suspension was warmed to 40°C, kept at this temperature for 2 h, and finally cooled to room temperature. The resin particles were filtered off and washed with water, 1 M hydrochloric acid, sodium hydrogen carbonate solution, water, ethanol, and chloroform successively. The washed amidoporphyrin resin (Catalyst A1) was dried and subjected to elemental analysis. 0.99% N was found. This value corresponds with the content of 0.12 mmol of amidoporphyrin moieties per g of resin.

2.1.2. Dicyclohexylcarbodiimide procedure [7]

The porphyrin (0.50 g, 0.71 mmol), the benzhydrylamine resin (5 g), 1-hydroxybenzotriazole (0.15 g, 1.1 mmol), and *N*-ethylmorpholine (1 ml) were dissolved in tetrahydrofurane (40 ml). The solution was stirred in an ice-water bath while dicyclohexylcarbodiimide (0.46 g, 2.2 mmol) was added. Stirring was continued for 2 h at 0°C and for an additional 12 h at room temperature. The resin particles were filtered off and washed with hot chloroform (50°C), 15% methanol in chloroform (50°C) and acetic acid (80°C) successively, and then with water, 1 M hydrochloric acid, sodium hydrogen carbonate solution, water, ethanol, and chloroform. The washed amidoporphyrin resin (Catalyst A2) was dried and subjected to elemental analysis. For A2, 0.84% N was found which resembles 1.10 mmol of amidoporphyrin moieties per g of resin.

2.1.3. Mixed anhydride method [8]

The porphyrin (0.50 g, 0.71 mmol) was suspended in chloroform (30 ml), redistilled triethylamine (1 ml) was added, the mixture was cooled in the ice-water bath, ethyl chloroformate (0.5 ml, 16.2 mmol) was added, and the whole was stirred for a period of 30 min. After that, the benzhydrylamine resin (5 g) was added to the mixture followed by the addition of precooled chloroform (30 ml) with a second portion of triethylamine (0.5 ml). The suspension was stirred under cooling on an ice bath for 30 min and then the temperature was allowed to rise to 20°C. After an additional 30 min of stirring the suspension was gently heated to 60°C and kept at this temperature for 2 h, and than cooled to room temperature. The resin particles were filtered off and washed with water, 1 M hydrochloric acid, sodium hydrogen carbonate solution, water, ethanol, and chloroform, successively. The washed amidoporphyrin resin (Catalyst A3) was dried and subjected to elemental analysis. For A3, 0.79% N was found which corresponds with the content of 0.09 mmol amidoporphyrin moieties per g of resin.

2.1.4. Capping procedure

The obtained amidoporphyrin resin was suspended in chloroform (30 ml) containing redistilled triethylamine (1 ml), the mixture was cooled in the ice-water bath, and pivaloyl chloride (0.5 ml, 4.0 mmol) was added. The reaction and its work-up were carried out following the acid chloride procedure. The elemental analyses of capped resins (B1, B2 and B3) showed only negligible differences in comparison with those of A1, A2 and A3. The reaction proceeded quantitatively (yield $> 99\%$), as confirmed by IR spectroscopy.

2.1.5. Metallation procedure

Catalysts (3 g) were converted to their Fe^{3+} and Mn^{3+} metallocomplexes under reflux in ethanol (30 ml) with the appropriate acetylacetonate complex (0.28 mmol) for 24 h. The metallated resins were thoroughly washed with ethanol (40–50°C), 50% aqueous ethanol, water, 80% aqueous acetic acid, and ethanol, and eventually dried in vacuum. The content of metal ion in metallated catalysts (C1, C2 and C3) (regular content of catalytic sites) was determined by atomic absorption spectroscopy after mineralization in concentrated perchloric acid, and was shown to be 0.122 mmol/g of resin in case of C1, 0.100 mmol/g of resin in case of C2 and 0.091 mmol/g of resin in case of C3.

2.2. General procedure for epoxidation experiments

In a typical experiment, the catalyst in the amount resembling to 5×10^{-4} mmol catalytical units (ca. 4–5 mg, according to the catalyst substitution density) was suspended in dichloromethane (7 ml) along with styrene (200 mg, 2.10 mmol) and pyridine (4 mg, 0.05 mmol) or imidazole (4 mg, 0.06 mmol), and to the thus formed solution the oxidizing agent, either iodosobenzene (500 mg, 2.27 mmol) or anhydrous *t*-butyl hydroperoxide in dichloromethane (1 ml, 2.22 mmol), was added. Reaction was followed by GC by taking-off the aliquots (0.5 ml), filtering them and submitting to analyses. Reaction products were determined by comparison of the retention times with those of the authentic samples and proved by GC–MS. The proportions of the products obtained were calculated from the total of all products (including unidentified ones) in the reaction mixture. The substrate conversion (percentage figures) were calculated from the amount of substrate submitted to the reaction.

3. Results and conclusions

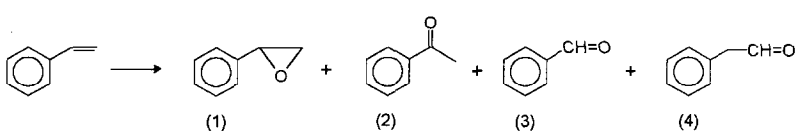
Epoxidation of styrene with iodosobenzene as oxidizing agent afforded a mixture of products according to the Scheme 1.

Styrene oxide (Product 1) was identified as the main component (82–90%) in the product mixture. Further products, representing 9–17% of the mixture, were identified as acetophenone (Product 2), benzaldehyde (Product 3) and 1-phenylacetaldehyde (Product 4). Under the use of pyridine as axial ligand the mutual ratio of the detected products 1, 2, 3, and 4 was found to be 100:3:6:4 for Mn^{3+} catalyst and 100:6:9:7 for Fe^{3+} catalyst while with the use of imidazole the respective figures for product composition were 100:2:5:4 for Mn^{3+} catalyst and 100:2:8:7 for Fe^{3+} catalyst. The reaction mixture contained some other minor products that were not identified and formed a mere 1–2% of the total. Table 1 shows the substrate conversion (sum of all products) after 24 h and the proportion of epoxide in the product mixture (both in %) depending on the axial ligand and the central ion used.

3.1. Porphyrins covalently bound to polystyrene, part II

Epoxidation of styrene with *t*-butyl hydroperoxide afforded a mixture of products, as in Scheme 1, in which styrene oxide (Product 1) was found to be the main product again (69–78%). Further products, a total of 18–24%, were identified as acetophenone (Product 2), benzaldehyde (Product 3), and 1-phenylacetaldehyde (Product 4). With the use of pyridine as axial ligand, the proportions of products 1, 2, 3, and 4 were found to be 100:5:18:11 for the Mn^{3+} catalyst and 100:7:23:14 for the Fe^{3+} catalyst, while under the use of imidazole the corresponding figures were 100:3:17:8 for the Mn^{3+} catalyst and 100:7:21:12 for the Fe^{3+} catalyst. The total amount of the non-identifiable products was less than 6% of the whole mixture. Table 2 shows the substrate conversion (sum of all products) after 24 h and the share of epoxide in the product mixture (both in %) depending on the axial ligand and the central ion used.

In order to find out the plot of substrate



Scheme 1. Epoxidation of styrene by iodosobenzene or t-butyl hydroperoxide.

Table 1
Epoxidation of styrene according to Scheme 1 mediated by amidoporphyrin–polystyrene catalysts (C2) using iodosobenzene after 24 h

Axial ligand	Pyridine		Imidazole	
Total conversion and epoxide yield (both in %)	Substrate conversion	Epoxide yield	Substrate conversion	Epoxide yield
Fe(3 +) catalyst	71	82	78	85
Mn(3 +) catalyst	83	89	89	90

conversion (per mmol of catalytic units) vs. density of saturation of the benzhydryl amine substitution of the resin we performed the experiments in which equimolar amounts of catalysts with varying density of resin substitution were used. It was found that at low densities of saturation of the substitution of the resin by catalytic centres, no significant changes of the stability during the course of the reaction of the catalyst took place. Only at the catalysts with a higher density of catalytic centres, some irreversible deactivation of certain amount of these centres takes place at the beginning of the experiment, probably as a result of the destruction of the less-protected centres situated on the surface of polymer particles. This is manifested by ca. 30% lowering of the catalytic efficiency in the course of the first 5 h of the experiment after which, however, the substrate conversion rate remains stable (as for the linear, stable rise). Table 3 shows, on a standard arrangement of the epoxidation experiment, the dependence of substrate conversion on the density of resin substitution.

The achieved results allow for drawing the following conclusions:

Preparation of catalytically active models of the monooxygenase systems based on the amidoporphyrin–polystyrene catalysts is simple, efficient and versatile (in the sense of finding several equally suitable variants of the preparation, according to the synthetic possibilities provided by the porphyrins intended for use as catalytic centres).

Catalytic systems based on the use of amidoporphyrin catalysts make use of the easily ac-

Table 2
Epoxidation of styrene according to Scheme 1 mediated by amidoporphyrin–polystyrene catalysts (C2) using *t*-butyl hydroperoxide after 24 h

Axial ligand	Pyridine		Imidazole	
Total conversion and epoxide yield (in %)	Substrate conversion	Epoxide yield	Substrate conversion	Epoxide yield
Fe(3 +) catalyst	98	69	99	71
Mn(3 +) catalyst	100	75	100	78

Table 3
Epoxidation of styrene by amidoporphyrin–polystyrene catalysts containing equimolar amount of catalytic sites using iodoso-benzene/imidazole system

	Total conversion of substrate after 24 h (in %)			
Catalyst prepared from	C1	C2	C3	CX
Density of substitution (mmol)	0.122	0.100	0.095	0.310
Fe(3 +) catalyst	79	78	79	61
Mn(3 +) catalyst	90	89	92	68

Catalyst CX was prepared using the acid chloride method from the benzhydryl amine resin of a 0.45 mmol/g amine substitution density.

cessible, high-yields-furnishing, unhindered *meso*-tetraphenylporphyrins, i.e., porphyrins without further substituents which in other cases contribute to enhance the stability of catalysts [1].

It turned out that the amide bond between porphyrin catalytic centres and the resin is sufficiently stable under conditions of the epoxidation reactions and allows to achieve a high turnover of the catalyst at the model reactions of this kind. The results achieved at the epoxidation experiments are comparable to those observed with the systems based on hindered metalloporphyrin catalysts. These results further support a general assumption on the possibility to model the role of apoprotein by making use of a neutral, sufficiently flexible environment which would enable to create suitable conditions for 'accommodation' of the porphyrin catalytic centres in the structure of the resin by bringing about the physico-chemical conditions similar to those existing in the active centres of the monooxygenase systems.

Acknowledgements

This work was supported by a grant of Howard Hughes Medical Institute International Research Scholars Program HHMI 75195-541101, by a grant of the Grant Agency of the Czech Republic GA ČR 500/009/94 and internal grant of AV ČR, No. 45535.

References

[1] B. Meunier, Chem. Rev. 92 (1992) 1411.
[2] P.R. Ortiz de Montellano ed., Cytochrome P-450 (Plenum Press, New York, 1985); A. Bassoli, G. Di Gregorio, B. Rinodone, S. Tollari and F. Chioccara, J. Mol. Catal. 53 (1989) 173; J. Bernadou, M. Bonnafous, G. Labat, P. Loiseau and B. Meunier, Drug. Metab. Dispos. 19 (1991) 360; S.M.S. Chauhan, T.S. Kohli, K. Visweswara and A. Gulati, Indian J. Chem. 29 B (1990) 539.
[3] A.W. van der Made, J.H.W. Smeets, R.J.M. Nolte and W.J. Drenth, J. Chem. Soc., Chem. Commun. (1983) 1204.
[4] P. Anzenbacher and V. Král, J. Mol. Catal. A 96 (1995) 311; L.D. Rollman, J. Am. Chem. Soc. 97 (1975) 2132; K. Maruyama, H. Tamiaki and S. Kawabata, J. Chem. Soc., Perkin. Trans. 2 (1986) 543; H. Uno, K. Takata and Y. Mizutani, React. Polymers 16 (1991/92) 297; Z.-L. Liu, J.-W. Huang and L.-N. Ji, J. Mol. Catal. A 104 (1996) L193.
[5] P. Anzenbacher, Jr., Thesis, Charles University, Prague, 1992; P. Anzenbacher, Jr., V. Král and J. Günterovà, unpublished results.
[6] R.G. Little, A.J.A. Loach and J.A. Ibers, J. Het. Chem. 32 (1967) 476.
[7] M. Bodanszky and A. Bodanszky, The Practice of Peptide Synthesis (Springer, Berlin, 1984).
[8] A.R. Tatchell et al., ed., Vogel's Textbook of Practical Organic Chemistry, 5th Ed. (Wiley, New York, NY, 1989).