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Imidazole versus pyridine as ligands for metalloporphine immobilization in ligninolytic peroxidases-like biomimetic catalysts

Paolo Zucca[a,b], Gianmarco Cocco[b], Stefania Manca[b], Daniela Steri[b], Enrico Sanjust[b,*]

[a] *Consorzio UNO (Consortium for University at Oristano), Oristano, Italy*
[b] *Dipartimento di Scienze Biomediche, Università di Cagliari, Italy*

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ABSTRACT

This paper describes the immobilization of 5,10,15,20-tetrakis(2,6-dichlorophenyl)porphine-manganese(III) through coordinative bond onto imidazolyl- and pyridyl-grafted silica, emulating the active site of ligninolytic peroxidases. To this purpose, plain silica gel was functionalized with two organosilanes: the first bearing an imidazolyl functionality, the other one bearing a pyridyl functionality. Manganese-porphine was then immobilized onto the two modified silicas. The two catalytic adducts have been fully characterized to point out the effect of the ligands on their catalytic features. Pyridine-immobilized metalloporphine was found to be a much more performant catalyst, giving significantly higher conversion rates with all the tested substrates: a lignin-model compound such as veratryl alcohol, the lignin peroxidase well-known substrate azure B, and recalcitrant textile dyes such as alizarin red S, phenosafranine, methylene blue, methyl green, xylenol orange, and methyl orange. Imidazole-immobilized metalloporphine on the contrary has proved to be more stable than its pyridine-based counterpart. Besides, the ligands seem to promote different catalytic pathways in the two adducts. These results allow giving rational and rigorous insights about the effect of the ligands during the immobilization of metalloporphines, thus helping in the design of catalysts with specific feature (i.e. stability, reactivity).

1. Introduction

Redox-active metalloporphines can use hydrogen peroxide or other suitable oxidants in several reactions, such as hydroxylation and epoxidation of hydrocarbons [1–3], as well as oxidation of phenols and aromatic alcohols [4–8], humic acids [9], sulfides [10], and textile dyes [11–13]. In particular, several electron-deficient metalloporphines have been synthetized (*second* and *third generation* metalloporphines [14]), showing promising catalytic features (chemical robustness, high conversion yields, stereo- and enantioselectivity, possibility of mild operational conditions, etc.) [14,15].

Their ability to emulate several enzymes has been also described, with particular emphasis on cytochrome P-450 [16] and ligninolytic peroxidases [15]. The emulation of the latter class of enzymes suggests their use in the detoxification of recalcitrant aromatic phenolic and non-phenolic substrates, such as lignocellulosic wastes, olive-mill wastewaters, textile dyes [15] etc. In this applicative perspective, heterogenization of catalysts is compulsory for economic concerns. Several approaches have been attempted, including adsorption, encapsulation, ion exchange and covalent binding (recently reviewed in [15]). However, real biomimesis of ligninolytic peroxidases can be accomplished only through immobilization via coordinative bond involving the central metal ion. In fact, in enzyme active sites the imidazole of a histidine residue coordinates ferri-heme. Such an approach led not only to proper structural biomimesis, but also to increased stability and activity of the catalysts [4,14,17].

Imidazole can be also replaced by pyridine as metalloporphine ligand. Although pyridine is not biomimetic, pyridyl function as a ligand for redox-active metalloporphines is of potential interest for practical applications. In fact, the stronger electron-withdrawing effect of pyridine in comparison with imidazole alters the overall behavior of the obtained catalysts, acting in particular on the redox potentials of the resting metalloporphine and on the catalytic

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Abbreviations: SG, silica gel; IPS, (3-[1-imidazolyl]-propylcarbamoyl)-3′-aminopropylsilica; PSG, 4-pyridyl-methyl-carbamoyl-aminopropylsilica; MnTDCPP, 5,10,15,20-tetrakis(2,6-dichlorophenyl)porphine-manganese(III); VA, veratryl alcohol; ARS, alizarin red S; AzB, azure B; PNS, phenosafranine; XO, xylenol orange; MG, methyl green; MB, methylene blue; MO, methyl orange; MnP, manganese peroxidase, E.C. 1.11.1.13; LiP, lignin peroxidase, E.C. 1.11.1.14.

* Corresponding author at: Dipartimento di Scienze Biomediche, Università di Cagliari, Cittadella Universitaria, 09042 Monserrato (CA), Italy.
Tel.: +39 070 675 4518; fax: +39 070 675 4527.
E-mail addresses: sanjust@unica.it, enrico.sanjust@gmail.com (E. Sanjust).

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intermediates. Therefore, metalloporphines immobilized via coordinative bond to pyridyl functionality show promising catalytic features [10,18,19].

In this paper, we describe two novel catalysts based on a *second generation* metalloporphine, 5,10,15,20-tetrakis(2,6-dichlorophenyl)porphine-manganese(III) (MnTDCPP). This macrocycle has been immobilized via coordinative bond onto two modified silica supports functionalized with imidazole (IPS) and pyridine (PSG) moieties (Fig. 1c and d). And the effects of the two ligands on metalloporphine catalytic activity have been studied, giving also mechanistic insights. To this purpose, several oxidant and reducing substrates were included in the study, to evaluate catalytic activity under some operational conditions of potential applicative interest. The role of Mn^{2+} as a redox mediator was also studied to discern between LiP- and MnP-like activity. Recently, a comparative study about the effect of these ligands in homogenous phase has been carried out [20]. While, for what in our knowledge, the present study is the first report of a rigorous comparison about the effect of imidazole and pyridine – as ligands for metalloporphine immobilization – toward the catalytic behavior of the same immobilized metalloporphine.

2. Experimental

All the reagents used were of the best grade available, and were used as purchased without further purification. Silica gel 100 (SG) came from Fluka (cat. no. 60746), 5,10,15,20-tetrakis(2,6-dichlorophenyl)porphine-manganese(III) (MnTDCPP) came from Porphyrin System (Lübeck, Germany, cat. no. PO890047), LiP (E.C. 1.11.1.14) was from Sigma–Aldrich (cat. no. 42603). Laccase from *Pleurotus sajor-caju* was purified through two chromatographic steps (details in [21,22]).

Spectrophotometric analyses were carried out with UltroSpec 2100 pro (Amersham Bioscience). Concentrations of substrates and products were also checked by UV–HPLC and GC–MS, using previously described methods [23,24].

2.1. Synthesis of IPS/MnTDCPP

IPS/MnTDCPP was synthesized as previously described [17], with minor modifications. Briefly, 10 g plain silica gel was functionalized by overnight reaction at 80 °C with 10 mmol 3-(1-imidazolyl)propylcarbamoyl-3′-aminopropyl-triethoxysilane (synthetized as described in [17], Fig. 1a) in 10 mL diglyme. The activated silica (IPS) was washed consecutively with 0.5 M HCl, H_2O, 0.1 M NaOH and H_2O again. The wet silica was then carefully dried overnight in a vacuum oven.

IPS (1 g) was then treated with 20 mg MnTDCPP, solubilized in 10 mL of DMSO, then the reaction mixture was kept at 25 °C in the dark under stirring overnight. After this, excess metalloporphine was washed away with DMSO and 2-propanol. The bound

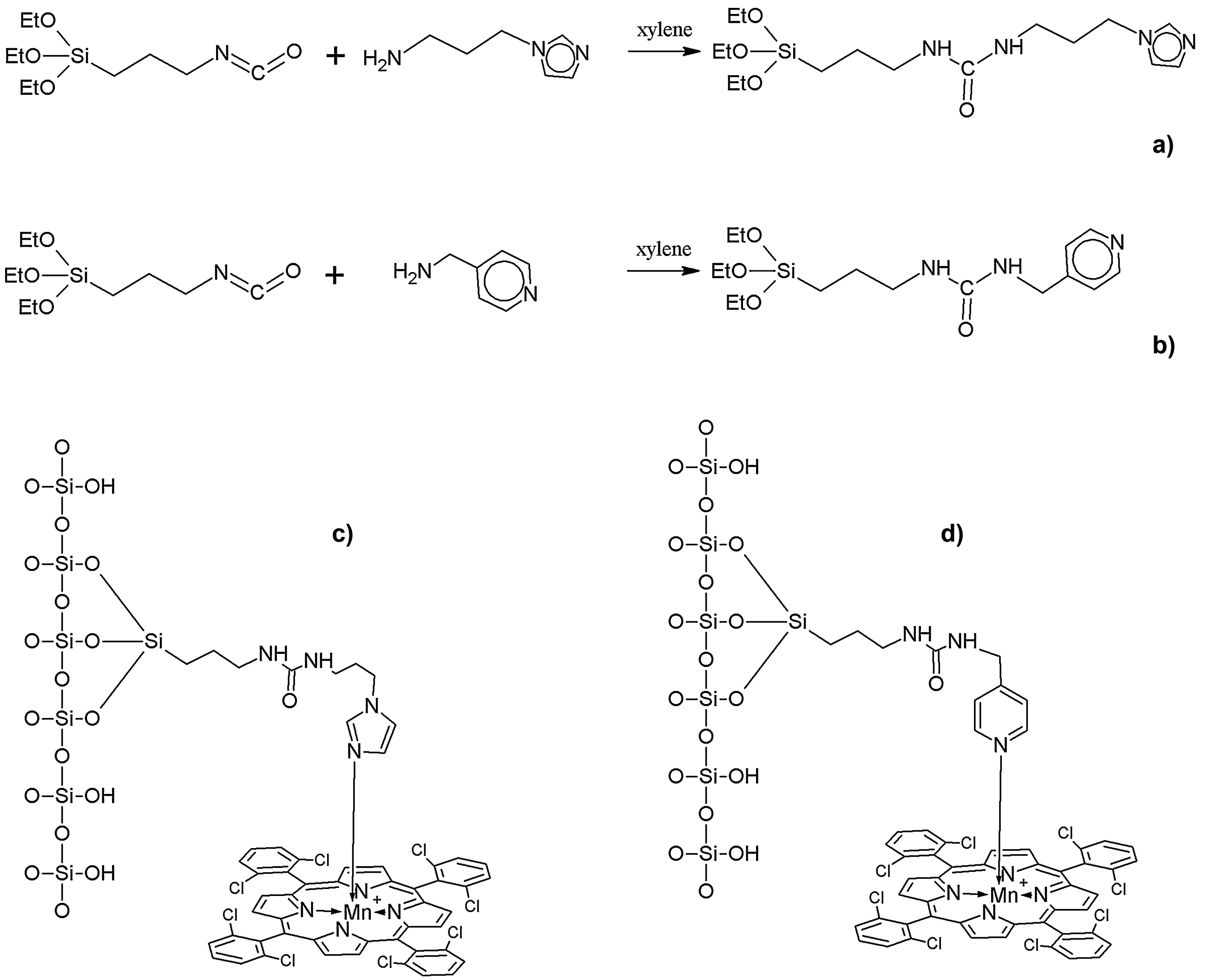

Fig. 1. Synthesis of (3-[1-imidazolyl]-propylcarbamoyl)-3′-aminopropylsilica (IPS, a) and 4-pyridyl-methyl-carbamoyl-aminopropylsilica (PSG, b). Chemical structures of the catalysts described in the present study: IPS/MnTDCPP (c) and PSG/MnTDCPP (d).