Tetrahedron 70 (2014) 4245–4249

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Manganese terpyridine artificial metalloenzymes for benzylic oxygenation and olefin epoxidation

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ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form 28 February 2014
Accepted 3 March 2014
Available online 12 March 2014

Keywords:
Artificial metalloenzyme
Manganese
C–H functionalization
Epoxidation
Biocatalysis

ABSTRACT

New catalysts for non-directed hydrocarbon functionalization have great potential in organic synthesis. We hypothesized that incorporating a Mn-terpyridine cofactor into a protein scaffold would lead to artificial metalloenzymes (ArMs) in which the selectivity of the Mn cofactor could be controlled by the protein scaffold. We designed and synthesized a maleimide-substituted Mn-terpyridine cofactor and demonstrated that this cofactor could be incorporated into two different scaffold proteins to generate the desired ArMs. The structure and reactivity of one of these ArMs was explored, and the broad oxygenation capability of the Mn-terpyridine catalyst was maintained, providing a robust platform for optimization of ArMs for selective hydrocarbon functionalization.

1. Introduction

New catalysts for non-directed hydrocarbon functionalization have great potential for applications in organic synthesis.[1,2] By avoiding the need for directing groups, these species expand the range of substrates on which they can act and eliminate synthetic steps and byproducts associated with directing group installation and removal.[3] A number of catalysts, including dirhodium tetracarboxylate complexes[4] and several different iron[5] and manganese[6,7] complexes, are particularly notable in this regard and are widely used for insertion of carbene,[8] nitrene,[8] and oxo[9] fragments into C–H bonds and olefins. While reactivity trends for such catalysts have been outlined, controlling their selectivity,[9] particularly on complex substrates,[1,2] remains difficult.

Crabtree and Brudvig have demonstrated that the selectivity of Kemp's-triacid-based Mn-terpyridine complexes for oxygenation of certain carboxylic acid-substituted substrates can be controlled by catalyst-substrate hydrogen bonding.[10–13] The reported examples illustrate the potential for supramolecular interactions to control the selectivity of Mn-terpyridine catalysts but require the use of a carboxylic acid directing group. We hypothesized that incorporating Mn-terpyridine cofactors into protein scaffolds would lead to artificial metalloenzymes (ArMs)[14] in which the selectivity of the Mn cofactor could be controlled by substrate binding to the protein. Mn-terpyridine complexes catalyze a number of oxygenation reactions,[15] ethereal α-C–H oxidation,[15] and hydrocarbon desaturation,[16] suggesting that the proposed ArMs could possess broad reaction scope. The utility of ArMs for selective catalysis has been demonstrated for a number of different reactions.[17] Hayashi recently demonstrated that myoglobin reconstituted with a Mn-porphycene cofactor catalyzes benzylic oxygenation,[18] but we are unaware of any reports of similar reactions using ArMs generated via covalent scaffold modification.[14] While biocatalysts (e.g., cytochromes P450) for this reaction exist, altering their substrate scope and site selectivity can be challenging.[19,20] The potential ease with which a Mn-terpyridine cofactor could be incorporated into different proteins[21] to modulate selectivity led us to develop a robust platform to explore the feasibility of this approach.

2. Result and discussion

Maleimide-substituted cofactor **3** was synthesized[22,23] from the known amine-substituted terpyridine, **1**[24,25] (Scheme 1).

Cysteine mutants of tHisF[26] and apo-nitrobindin (Nb)[27] were then prepared as scaffolds for initial bioconjugation studies. Both of these proteins have been used as ArM scaffolds by us[28] and by others[22,29] and therefore serve as ideal substrates for initial studies on cofactor bioconjugation using **3**. Reacting tHisF-C48 with **3** provided modest conversion (ca. 40%) to tHisF-C48-**3** within 2 h (Table 1, entry 1), but longer reaction times appeared to give multiple addition products (ESI-MS, data not shown), a common problem with maleimide bioconjugation reactions.[30] On the other

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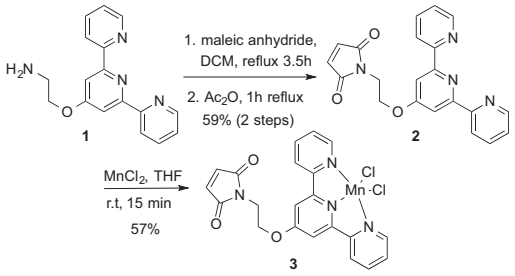
0040-4020/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2014.03.008



Scheme 1. Synthesis of cofactor **3**.

Table 1
Mass spectrometry and conversion data for ArMs

Entry	Scaffold (MW)[a]	Cofactor (MW)[b]	MW_{ArM}[a]	MW_{obs}[c]	Conv. (%)[c]
1	tHisF-C48 (28,754)	**3** (372)	29,126	29,128	40
2	tHisF-C48A50 (28,712)	**3** (372)	29,084	29,082	85
3	Nb-C96 (19,490)	**3** (372)	19,862	19,862	90
4	Nb-C125 (19,547)	**3** (372)	19,919	19,919	95

[a] Protein MW calculated by using tools at http://www.scripps.edu/~cdputnam/protcalc.html.
[b] The $MnCl_2$ fragment not detected.
[c] Observed MW and approximate conversion from ESI-MS after 2 h reaction.

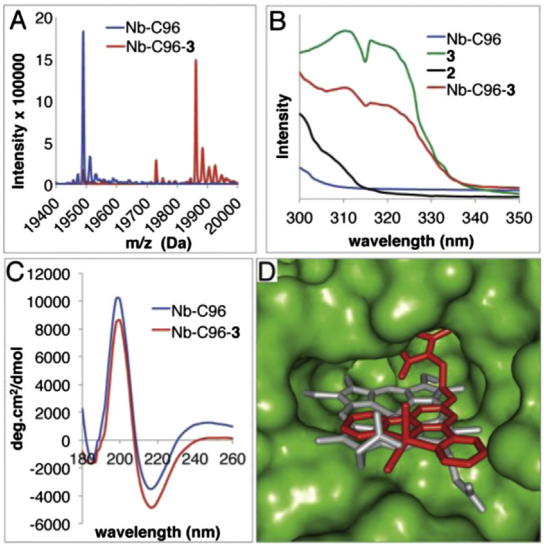


Fig. 1. (A) HR ESI-MS spectra of Nb-C96 and Nb-C96-**3** showing the efficiency of Nb bioconjugation. (B) UV spectra of Nb-C96, Nb-C96-**3**, **2**, and **3** showing the presence of $MnCl_2$ in Nb-C96-**3**. (C) CD spectra of Nb-C96 and Nb-C96-**3** showing proper Nb folding following bioconjugation. (D) Overlay of a DFT-optimized structure of **3** (red) covalently linked (Pymol) to a crystal structure of Nb (PDB ID 3EMM[27]) and the native heme (gray).

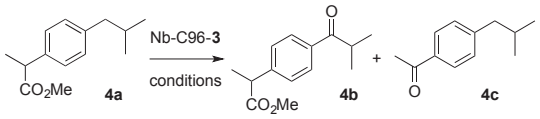
hand, we found that mutating a leucine residue (L50) located directly above C48 in the β-barrel of tHisF[26] led to a marked increase in bioconjugation rate and selectivity, and over 80% conversion to tHisF-C48A50-**3** was achieved within 2 h (entry 2). These improvements presumably resulted from decreased steric hindrance proximal to the cysteine nucleophile.

Nitrobindin is a heme protein with a β-barrel structure and a large hydrophobic pocket for heme binding. Cysteine mutations at suitable locations within the β-barrel can be used to position cofactors within the heme binding pocket of the apo enzyme (Nb).[29] Bioconjugation of two cysteine mutants of Nb, C96 within the β-barrel and C125 on the protein surface, were examined, and both of these provided greater than 90% conversion to the corresponding Nb-C-**3** bioconjugates (entries 3 and 4). These results, in addition to those noted above using tHisF, highlight two key advantages of covalent bioconjugation for ArM formation:[21] the nature of both the scaffold itself and the local environment of the bioconjugation site can be readily manipulated to facilitate cofactor installation.[14]

HR ESI-MS, CD spectroscopy, and UV–vis spectroscopy (Fig. 1), the reactivity of this ArM under a variety of conditions was explored to examine the impact of confining the Mn center within the heme binding pocket. Benzylic oxygenation of ibuprofen methyl ester (**4a**) was initially investigated based on previous reports from Crabtree and Brudvig.[13] As previously noted, these researchers reported that ibuprofen (**5a**) oxygenation can be catalyzed by Kemp's-triacid-based Mn-terpyridine complexes to provide a mixture of **5b** and **5c**, the latter resulting from decarboxylation following oxygenation (Table 3). The selectivity of this reaction could be controlled using hydrogen bonding between carboxylic acid groups on ibuprofen and the catalyst, with the presence of the carboxylic acid on the catalyst improving the **5b**:**5c** ratio from 77:23 to 98.5:1.5.

We found that similar benzylic oxygenation and oxygenation/decarboxylation of **4a** was catalyzed by Nb-C96-**3** (Table 2). Oxone, peracids, and a number of additional oxidants are known to promote Mn-terpyridine catalyzed oxygenation, and both oxone and peroxyacetic acid proved to be compatible with Nb-C96-**3**. Crucial to this reactivity, however, was the use of phosphate buffer (Table 2, entries 1–3). A modest excess of peroxyacetic acid was sufficient to achieve maximum conversion, and this helped to minimize pH changes over the course of the reaction (entries 3–5). While the Nb scaffold can tolerate 20% v/v acetonitrile, greater than this amount led to protein precipitation and dramatically reduced conversion. Maximum conversion was observed using 10% v/v acetonitrile, and omitting acetonitrile entirely led to reduced conversion, presumably due to poor substrate solubility (entries 6–8). Conducting the reactions at room temperature or decreasing ArM loading to 2.5 mol % both led to significant decreases in conversion (entries 9–10). All reactions in Table 2 provided a similar **4b**:**4c** ratio of 88:12. Nb-C125-**3** provided this same ratio with slightly higher conversion, suggesting that improved substrate access to **3** attached to the protein surface (in Nb-C125-**3**) offset any potential scaffold acceleration[31] afforded by the heme binding pocket (in Nb-C96-**3**).

Table 2
Optimization of ArM catalyzed oxidation[a]



Entry	Solvent	Oxidant	Conv.[b] (%)
1	Tris (25 mM, 7.0), 20%ACN	Oxone, 10 equiv	0
2	NaPi (100 mM, 7.0), 20%ACN	Oxone, 10 equiv	77
3	NaPi (100 mM, 7.0), 20%ACN	AcOOH, 10 equiv	85
4	NaPi (100 mM, 7.0), 20%ACN	AcOOH, 2.5 equiv	83
5	NaPi (100 mM, 7.0), 20%ACN	AcOOH, 2 equiv	76
6	NaPi (100 mM, 7.0), 30%ACN	AcOOH, 2.5 equiv	12[e]
7	NaPi (100 mM, 7.0), 10% ACN	AcOOH, 2.5 equiv	91
8	NaPi (100 mM, 7.0)	AcOOH, 2.5 equiv	24
9[c]	NaPi (100 mM, 7.0), 10% ACN	AcOOH, 2.5 equiv	45[e]
10[d]	NaPi (100 mM, 7.0), 10% ACN	AcOOH, 2.5 equiv	58

[a] Reaction conditions: 1 mM substrate, 5% ArM, 4 °C, overnight in dark.
[b] Determined by GC analysis of DCM extract.
[c] Reaction conducted at room temperature.
[d] 2.5% ArM loading used.
[e] Protein precipitation was observed.