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Construction of a hybrid biocatalyst containing a covalently-linked terpyridine metal complex within a cavity of aponitrobindin

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ABSTRACT

A hybrid biocatalyst containing a metal terpyridine (tpy) complex within a rigid β-barrel protein nitrobindin (NB) is constructed. A tpy ligand with a maleimide group, *N*-[2-([2,2′:6′,2″-terpyridin]-4′-yloxy)ethyl]maleimide (**1**), was covalently linked to Cys96 inside the cavity of NB to prepare a conjugate NB–**1**. Binding of Cu^{2+}, Zn^{2+}, or Co^{2+} ion to the tpy ligand in NB–**1** was confirmed by UV–vis spectroscopy and ESI–TOF MS measurements. Cu^{2+}-bound NB–**1** is found to catalyze a Diels–Alder reaction between azachalcone and cyclopentadiene in 22% yield, which is higher than that of the Cu^{2+}–tpy complex without the NB matrix. The results suggest that the hydrophobic cavity close to the copper active site within the NB scaffold supports the binding of the two substrates, dienophile and diene, to promote the reaction.

1. Introduction

Development of a hybrid biocatalyst combining a metal complex and a protein scaffold is a worthy objective because the scaffold would be expected to provide a well-defined and tunable environment for the metal complex [1–9]. Over the last decade, enantio- or chemoselective hydrogenations, Diels–Alder reactions, oxidations, cyclopropanations, and hydrolysis reactions have been demonstrated to be promoted by a series of hybrid biocatalysts [10–23]. The coordination sphere of the metal catalyst in the protein scaffold has been rationally designed and engineered to improve the catalytic activity and selectivity of the catalyst, which is occasionally supported by computational modeling and the selection strategy [24–26]. The intriguing features of hybrid biocatalysts have encouraged us to explore further promising combinations of synthetic metal complexes and biomolecular scaffolds.

Our group has focused on hybrid biocatalysts containing a covalently-linked synthetic metal complex inside nitrobindin (NB). NB is a small protein (18 kDa) and has a rigid β-barrel structure with a hydrophobic cavity containing a native heme cofactor. This cavity therefore provides a suitable platform to accommodate a hydrophobic metal catalyst and substrates when the heme cofactor is removed [27–29]. For example, a regioselective acetylene polymerization reaction was found to be promoted by a Rh complex-linked NB hybrid, where a Rh complex is covalently linked inside the β-barrel cavity of apoNB. Furthermore, the engineering of the cavity of the Rh-complex-linked protein by computationally-guided site-directed mutagenesis resulted in the identification of key residues that modulate and improve the *cis*/*trans*-stereoselectivity for the phenylacetylene polymerization reaction [29,30]. The results provide significant insights into the importance of designing the second coordination sphere consisting of protein matrices to improve the reaction selectivity.

As a next target, we constructed a hybrid biocatalyst containing a metal terpyridine (tpy) complex within the rigid β-barrel structure of NB (Fig. 1). The tpy ligand was selected because various metal tpy complexes have been studied in a broad range of chemical transformations such as cyclopropanation, cross coupling, and click reactions in combination with metal ions [30]. For example, Lewis and coworkers reported a Mn-tpy complex linked to NB which catalyzes benzylic oxygenation and olefin epoxidation [31]. In this work, we prepared tpy-modified NB (NB–**1**) and investigated the binding of metal ions, Cu^{2+}, Zn^{2+} and Co^{2+}. The catalytic activity toward the Diels–Alder reaction between azachalcone and cyclopentadiene was investigated for the hybrid biocatalyst NB–**1**/M^{2+}, where M^{2+} is Cu^{2+}, Zn^{2+}, or Co^{2+}.

2. Experimental section

2.1. Instruments

^{1}H NMR spectra (400 MHz) were recorded on a Bruker DPX400 NMR spectrometer. ^{1}H NMR chemical shift values are reported in ppm relative to the residual solvent resonances. CD spectra were measured by a JASCO J-820 circular dichroism spectropolarimeter. ESI–TOF MS analysis was performed by a Bruker micrOTOF Focus II mass spectrometer. MALDI–TOF MS analysis was performed by a Bruker Autoflex III Smartbeam mass spectrometer. UV–vis spectra were measured by a Shimadzu UV-3150 UV–VIS-NIR spectrophotometer. The pH values

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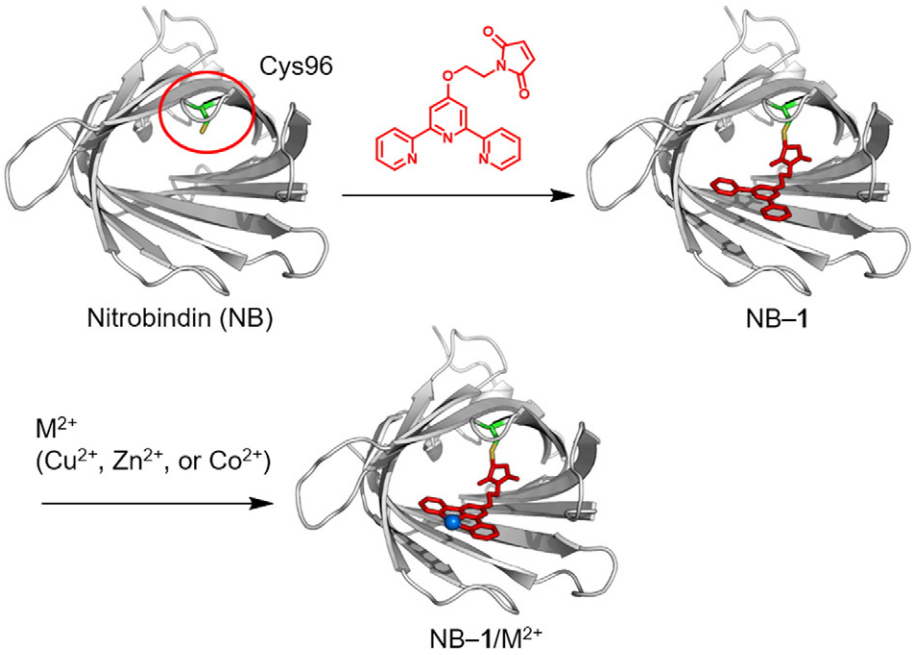


Fig. 1. Construction of terpyridine-modified nitrobindin NB–**1**/M^{2+}.

were determined by an F-52 Horiba pH meter. HPLC was performed on a Shimadzu HPLC system consisting of an LC-20AP solvent delivery module, a CTO-20A column oven, an SPD-M20A photodiode array UV–vis detector and a CBM-20A system controller using a CHIRALPAK IA-3 column (DAICEL).

2.2. Materials

All reagents and solvents were obtained from commercial sources and used without further purification. Ultrapure water was demineralized by a Merck Milli-Q integral 3 system.

2.3. Synthesis of a tpy ligand with a maleimide group

The maleimide-modified tpy ligand, *N*-[2-([2,2′:6′,2″-terpyridin]-4′-yloxy)ethyl]maleimide (**1**), was synthesized as shown in Scheme 1. 2-([2,2′:6′,2″-terpyridin]-4′-yloxy)ethylamine (**3**) and *N*-methoxycarbonylmaleimide (**4**) were synthesized according to the reported methods [32,33].

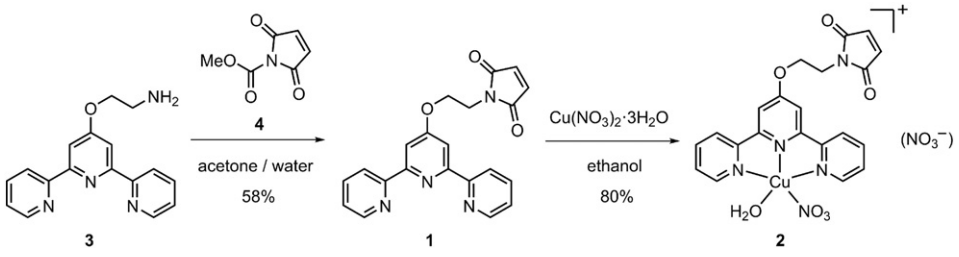
2-([2,2′:6′,2′′-terpyridin]-4′-yloxy)ethylamine (**3**) (290 mg, 1.0 mmol) in 40 mL of acetone/water (2/1) was added to a solution of $NaHCO_3$ (440 mg, 5.3 mmol) and *N*-methoxycarbonylmaleimide (**4**) (470 mg, 3.0 mmol) at 4 °C, and the reaction mixture was stirred for 1 h. The solution was added to water (6 mL) and stirred for 2 h at room temperature. The obtained precipitate was collected and washed with water. The product was recrystallized from acetone/water (4/1). Yield: 220 mg (58%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, J = 4.8 Hz, 2 H), 8.61 (d, J = 8.0 Hz, 2 H), 8.11 (s, 2 H), 7.97 (dd, J = 8.0, 7.5, 1.9 Hz, 2 H), 7.44 (dd, J = 7.5, 4.8 Hz, 2 H), 6.75 (s, 2 H), 4.50 (t, J = 5.6 Hz, 2 H), 4.05 (t, J = 5.6 Hz, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.34, 166.57, 156.78, 155.53, 148.71, 137.21, 134.26, 124.01, 121.54, 107.59, 64.86, 36.94.

2.4. Synthesis of Cu^{2+}-tpy complex **2**

Synthesis of Cu^{2+}-tpy complex **2** is shown in Scheme 1. Tpy ligand **1** (215 mg, 0.58 mmol) dissolved in 20 mL of EtOH was added to $Cu(NO_3)_2 \cdot 3H_2O$ (160 mg, 0.66 mmol) in 20 mL of EtOH and the mixture was stirred for 1 h at room temperature. The precipitate was collected, washed with EtOH, and dried in vacuo to give the Cu^{2+}-tpy complex **2**, [Cu(**1**)$(NO_3)_2$]. Yield: 260 mg (80%); anal. calcd for $C_{21}H_{16}CuN_6O_9$: C, 45.05; H, 2.88; N, 15.01. Found: C, 44.61; H, 3.07; N, 15.01.

2.5. Molecular structure of Cu^{2+}-tpy complex **2**

A single crystal of the Cu^{2+}-tpy complex **2** for X-ray structural analysis was obtained by recrystallization from hot water. Intensity data were collected with a Bruker D8 goniometer equipped with an APEX CCD area detector and an Incoatec microsource (Mo-α radiation, λ = 0.71073 Å, multilayer optics) at 100 K (Oxford Cryostream 700 instrument). The crystallographic data are shown in Table S1. The structure was solved by the direct method and expanded using SIR92 [34].



Scheme 1. Syntheses of tpy ligand **1** and Cu^{2+}-tpy complex **2**.