Pharmaceuticals, Functional materials, Green Chemistry

Construction of artificial metalloenzymes for challenging chemical transformations

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Abstract

This study demonstrates a redox engineering strategy to precisely control the catalytic reactivity of myoglobin (Mb) through rational cofactor substitution and electronic modulation. The native heme was systematically replaced with artificial iron porphyrins containing one or two trifluoromethyl groups at the β-positions (Figure 1), enabling broad tuning of Fe(II)/Fe(III) redox potentials. Among the engineered variants, Mb reconstituted with FePor$(CF_3)_2$ exhibited a markedly positive potential and demonstrated exceptional carbene transfer activity toward both aliphatic and internal alkenes–substrates generally unreactive in natural hemoproteins (Fig. 2). Spectroscopic, crystallographic, and kinetic studies revealed that electron-withdrawing CF_3 groups accelerate the formation of highly reactive radical-type intermediates responsible for the enhanced reactivity. These results highlight that redox modulation of the metal center is a powerful strategy to expand the catalytic repertoire of hemoproteins.

Background & Results

Heme proteins such as Mb catalyze a wide variety of oxidative and reductive reactions, but their catalytic efficiency and selectivity are usually improved through protein mutagenesis. Such approaches, however, often provide limited control over the electronic properties of the active site. To achieve direct tuning of redox reactivity, this study explores the substitution of the native heme with artificial cofactors having distinct electronic characteristics. Four Mb variants–native heme, FePorCF_3, FePor$(CF_3)_2$, and FePc–were prepared and their Fe(II)/Fe(III) redox potentials were found to range from +147 mV to −198 mV versus NHE. rMb(FePor$(CF_3)_2$) exhibited the most positive potential and showed superior catalytic performance in carbene transfer reactions, efficiently catalyzing the cyclopropanation of both aliphatic and internal alkenes with high turnover numbers and selectivity. Mechanistic studies including Hammett correlations, kinetic analyses, and spin-trapping experiments revealed that rMb(FePor$(CF_3)_2$) proceeds through a radical-type mechanism, while rMb(FePc) involves an electrophilic carbene pathway. These results clearly demonstrate that the redox potential of the cofactor is a key determinant of intermediate character and overall catalytic behavior in engineered hemoproteins.

Significance of the research and Future perspective

This research establishes a new design strategy for artificial metalloenzymes based on precise electronic control via cofactor engineering rather than conventional protein mutation. By redox tuning of metal cofactors, catalytic properties can be enhanced, diversified, and extended to promote non-natural chemical transformations that are beyond the scope of native enzymes. The rMb(FePor$(CF_3)_2$) system demonstrates high activity, stability, and oxygen tolerance, highlighting its potential for sustainable and practical catalysis. This redox-focused approach opens broad opportunities for hydrocarbon functionalization, CO_2 reduction, and photoredox chemistry. It provides a fundamental framework for developing next-generation bioinspired catalysts that unite enzymatic selectivity with synthetic versatility.

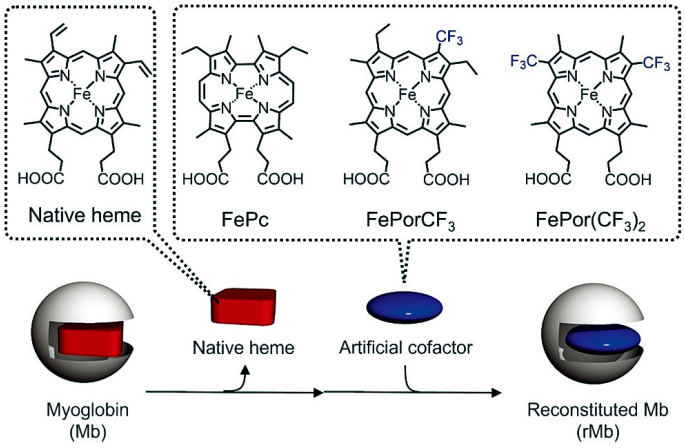

Figure 1 Schematic representation of reconstituted myoglobin with artificial cofactors and structures of the artificial cofactors

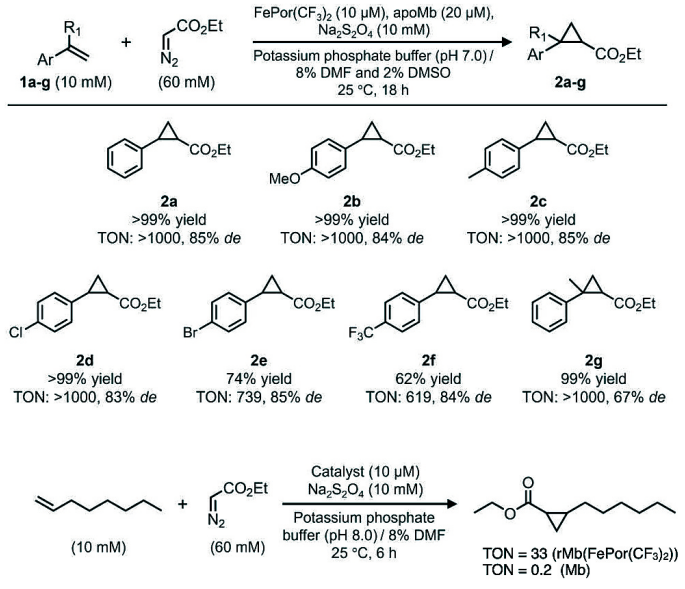

Figure 2 (Top)Yield and TON(turnover number) for cyclopropanation of styrenederivatives (bottom) TON for cyclopropanation of 1-octene

Patent

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Keyword artificial metalloenzyme design, cofactor engineering, redox-controlled catalysis