C–H Bond Amination Catalyzed by Engineered Hemoprotein Containing Iron Porphycene as an Artificial Cofactor

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Insertion of metal nitrene into a C–H bond is a powerful transformation to directly form a new C–N bond. Recently, artificial metalloenzymes based on hemoproteins have been reported as promising biocatalysts for nitrene transfer reactions.[1] Our group has previously reported C–H bond hydroxylation[2] and olefin cyclopropanation[3] catalyzed by myoglobin reconstituted with metal complexes of porphycene, a constitutional isomer of porphyrin. In this work, we demonstrate myoglobin containing iron porphycene as an artificial cofactor catalyzes C–H bond amination (Fig. 1).

We initially evaluated the intramolecular amination of 2,4,6-triisopropylbenzenesulfonyl azide catalyzed by reconstituted myoglobin with iron porphycene (rMb-FePc), native myoglobin (nMb) and iron porphycene (FePc). rMb-FePc exhibits higher turnover number (TON = 318) compared to nMb (TON = 255) and FePc (TON = 256). Interestingly, rMb-FePc yields the product by C–H bond amination with higher selectivity (96%) against competitive reduction of the azide substrate relative to nMb (80%). In addition, TON by rMb-FePc reached to 5.7 x 10^4 in the presence of a large excess of substrate. Kinetic experiments revealed that k_{cat}/K_m value of rMb-FePc (59 $mM^{-1} \cdot s^{-1}$) is 5-fold higher than that of nMb (12 $mM^{-1} \cdot s^{-1}$). This obvious difference of the k_{cat}/K_m values is derived from the k_{cat} values: k_{cat} of rMb-FePc and nMb are 55 s^{-1} and 14 s^{-1}, respectively. Furthermore, several rMb-FePc mutants were prepared and the catalytic reaction for 2,4,6-triethylbenzenesulfonyl azide was evaluated. The H64A mutant promotes the reaction with TON of 25 with 28%*ee*, whereas rMb-FePc and nMb show lower activities (TON = 6 and 2, respectively) with no enantioselectivity. This work represents that the incorporation of a suitable cofactor into protein matrices will be a useful strategy to develop new biocatalysts. Further investigations using other hemoproteins with FePc toward the C–H bond amination is now in progress.

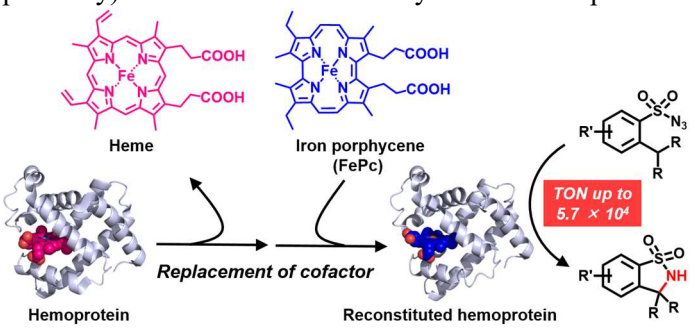

Fig. 1. Catalytic C–H bond amination by reconstituted hemoprotein.

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