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Chemoselective, Enzymatic C−H Bond Amination Catalyzed by a Cytochrome P450 Containing an Ir(Me)-PIX Cofactor

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Supporting Information

ABSTRACT: Cytochrome P450 enzymes have been engineered to catalyze abiological C−H bond amination reactions, but the yields of these reactions have been limited by low chemoselectivity for the amination of C−H bonds over competing reduction of the azide substrate to a sulfonamide. Here we report that P450s derived from a thermophilic organism and containing an iridium porphyrin cofactor (Ir(Me)-PIX) in place of the heme catalyze enantioselective intramolecular C−H bond amination reactions of sulfonyl azides. These reactions occur with chemoselectivity for insertion of the nitrene units into C−H bonds over reduction of the azides to the sulfonamides that is higher and with substrate scope that is broader than those of enzymes containing iron porphyrins. The products from C−H amination are formed in up to 98% yield and ∼300 TON. In one case, the enantiomeric excess reaches 95:5 er, and the reactions can occur with divergent site selectivity. The chemoselectivity for C−H bond amination is greater than 20:1 in all cases. Variants of the Ir(Me)-PIX CYP119 displaying these properties were identified rapidly by evaluating CYP119 mutants containing Ir(Me)-PIX in cell lysates, rather than as purified enzymes. This study sets the stage to discover suitable enzymes to catalyze challenging C−H amination reactions.

Cytochrome P450 enzymes (P450s) are native metalloproteins containing Fe-protoporphyrin IX (Fe-PIX) cofactors that catalyze the oxidations of C−H bonds in a wide range of substrates with exquisite selectivities.[1,2] In contrast, natural enzymes that catalyze C−H bond amination reactions are rare, and they react with narrower scope and lower selectivity.[3]

Recently, protein engineering and directed evolution of Fe-PIX enzymes has created variants that catalyze the intramolecular amination of C−H bonds with good enantioselectivity,[4−6] but the yields of the amination products are limited by poor chemoselectivity. These reactions form mixtures of products from insertion of the nitrene units into the C−H bonds and reduction of the sulfonyl azides to the sulfonamides. The products from reduction form in yields that are comparable to or greater than those of the products from nitrene insertion into C−H bonds in all cases.[7−10]

Recently, we described an efficient method for the reconstitution of apo-(PIX)-proteins with various abiological metal complexes of protoporphyrin and mesoporphyrin IX.[11,12] The reactivity of these artificial PIX proteins is distinct from that of the analogous Fe-containing heme proteins, affording abiological reactivity.[11,12] We hypothesized that some of the P450 proteins containing non-native metals would catalyze the reaction of sulfonyl azides with chemoselectivity for C−H bond amination over azide reduction that is higher than that of the P450s containing iron in the active site. Many metal porphyrin complexes are reported to catalyze the amination of C−H bonds with sulfonyl azides, although such reactions are often conducted above room temperature.[13−15] To enable the use of prospective artificial metalloenzymes at elevated temperatures, we focused on the hybrid catalysts generated from the protein CYP119,[12,16] which is a thermally stable P450 from the archaeon *Sulfolobus solfataricus*.

Here we report that variants of CYP119 containing an Ir(Me)-PIX cofactor catalyze the insertion of nitrenes into C−H bonds with greater than 20:1 chemoselectivity for insertion over reduction of the sulfonyl azide to the sulfonamide. These insertions into C−H bonds occur in yields up to 98% and TON up to ∼300. In one case, the enantiomeric excess reaches 95:5 er, and the reactions can occur with divergent site selectivity. The high activity of Ir(Me)-PIX CYP119 enzymes enables the formation of benzo-fused sulfamates via C−H amination, which have not been formed by Fe-PIX enzymes.

We commenced studies to create an artificial enzyme for chemoselective C−H amination by assessing the reactivity of a set of free metalloporphyrins IX (M-PIX) for the model reactions to convert sulfonyl azides **1** and **2** into sultams **3** and **4** under aqueous conditions at room temperature (Figure 1). These model azides would undergo aminations of C−H bonds catalyzed by the metal-porphyrin complex by forming a metal-nitrene complex, followed by insertion of the nitrene unit into either a tertiary or secondary C−H bond, respectively. The metalloporphyrin PIX complexes containing Fe, Cu, and Mn either did not react with the sulfonyl azides or reacted with

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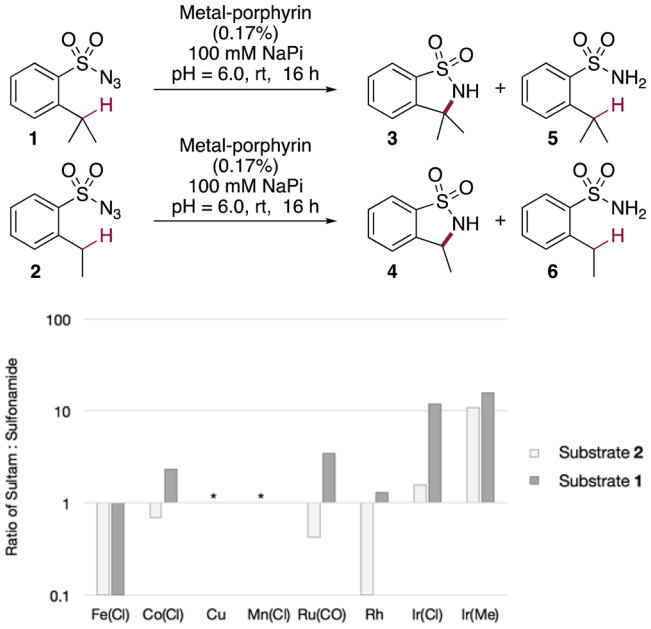

Figure 1. Reactions of sulfonyl azides **1** and **2** in the presence of a set of [M]-PIX-IX complexes, the putative catalysts for C−H amination reactions, to form the C−H insertion sultam products **3** and **4** and the sulfonamide reduction products **5** and **6**. The figure depicts the chemoselectivity for the formation of the C−H insertion products **3** and **4** over the reduction products **5** and **6** in the presence of each metal complex under aqueous conditions at room temperature. The bars reflect the molar ratio of the two products (sultam and sulfonamide) formed, and a comparison of the outcomes from insertions into a secondary C−H bond (substrate **1**, dark gray bars) and into a tertiary C−H bond (substrate **2**, light gray bars) is depicted. *Reactions catalyzed by Cu and Mn porphyrins produced trace sulfonamide product and no observable sultam.

chemoselectivity that strongly favored formation of sulfonamide products **5** and **6** over the sultam products **3** and **4**. Reactions of sulfonyl azide **1** in the presence of the metal-PIX complexes containing Co, Ru, or Rh preferentially formed sultam **3** over the sulfonamide product **5** with modest chemoselectivity. However, the reactions of sulfonyl azide **2**, containing stronger, secondary C−H bonds, catalyzed by the same complexes formed predominantly sulfonamide **6** over sultam **4**.

Although porphyrins containing iridium have not been reported to catalyze the insertion of nitrenes into C−H bonds,[17] we found that Ir(Me)-PIX is the most active and the most chemoselective catalyst among the series of M-PIX complexes we tested for the formation of products from C−H amination under aqueous conditions at room temperature. The selectivity of Ir(Me)-PIX for formation of the sultam over the sulfonamide was >10:1 for both substrates (Figure 1). In the presence of Ir(Me)-PIX, substrate **1** reacted to form sultam **3** in 92% yield, with 14:1 selectivity for formation of **3** over formation of sulfonamide **5**. Under otherwise identical conditions, substrate **2** containing secondary C−H bonds, which are less reactive than the tertiary C−H bond of **1**, underwent the amination reaction to form **4** in 72% yield, along with 6% of side-product **6**. The reaction of substrate **2** conducted at 37 °C occurred to form sultam **4** in 89% yield and sulfonamide **6** in 8% yield.

On the basis of these results, we used Ir-PIX as the cofactor in artificial P450s formed from CYP119, which were identified previously to be thermally stable,[12] as catalysts for C−H amination reactions (Figure 2). To assess initially the ability of Ir(Me)-PIX CYP119 variants to catalyze the insertion of

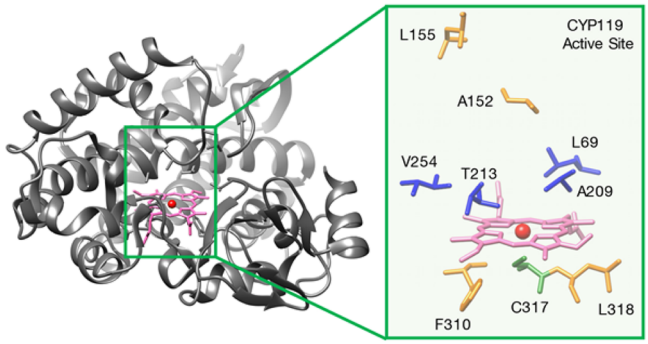

Figure 2. Structure of WT Fe-CYP119 (picture prepared in UCSF Chimera from PDB 1IO7). Left: Structure of Fe-CYP119. Right: Residues targeted in the evolution of the protein scaffold to increase activity and selectivity in C−H amination reactions.

nitrenes into C−H bonds, we evaluated reactions of sulfonylazide **2** to form sultam **4** in the presence of the wild-type Ir(Me)-PIX CYP119 enzyme, the variant containing the single mutation C317G to the axial ligand, and the variant 'CYP119-Max-L155G' (Figure 3). CYP119-Max-L155G, which

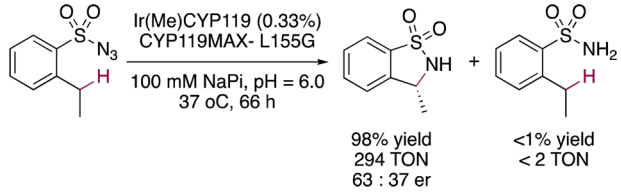

Figure 3. C−H amination reaction catalyzed by a variant of Ir(Me)-PIX CYP119 containing the following mutations: C317G, L69V, T213G, V254L, L155G. Selectivity and yield determined by SFC using an internal standard.

contains five mutations (C317G, T213G, L69V, V254L, L155G), was identified previously to be highly active for the insertion of carbenes into C−H bonds.[12] Although the reactivity of the WT and C317G Ir(Me)-PIX CYP119 enzymes was low, the reaction catalyzed by the variant CYP119-Max-L155G formed sultam **4** in high yield (98% yield, 294 TON) with excellent chemoselectivity (<1% yield of sulfonamide **6**). This result demonstrates that changing the metal site in a P450 from native iron to the abiological iridium-methyl unit creates an active and distinctly chemoselective catalyst for C−H amination.

The variant CYP119-Max-L155G formed sultam **4** in high yield (98% yield), but with a modest 63:37 enantiomeric ratio (er). To improve the enantioselectivity of this reaction, we created a library of plasmids encoding variants of CYP119 containing mutations at a subset of eight different positions within the active site of the enzyme (Figure 2).

In prior studies on this type of artificial metalloenzyme,[11,12] we purified each variant of the Ir(Me)-PIX enzyme prior to evaluating its reactivity. To accelerate the evaluation of mutant enzymes, we developed an approach to conduct directed evolution of artificial heme proteins that does not require the purification or concentration of the enzyme variants. By this approach, the apo forms of the CYP119 variants were overexpressed in *E. coli*. After lysis of the cells, removal of the

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cell debris, and dialysis into tris buffer, the Ir(Me)PIX cofactor was added to the cell lysate. The resulting solutions containing unpurified, reconstituted Ir(Me)-CYP119 mutants were used in catalytic experiments. By this protocol, we evaluated as catalysts 142 different Ir(Me)-PIX CYP119 systems that contained 2−4 mutations at the positions shown in Figure 2. Many of the mutants evaluated were inactive; however, several variants did form product **4** in an enantioselective fashion (Table S5). Specifically, the mutant C317G, T213G, V254L, F310L formed product **4** with 84:16 er, while the mutant C317G, T213A, A152L formed the opposite enantiomer of the product with 26:74 er.

The mutants that formed **4** with the highest enantiomeric ratios by this protocol were subsequently evaluated as purified enzymes for the same reaction of **2** to form **4** and in the reactions of similar sulfonyl azides to form products **7**, **8**, and **11** (Figure 4). These experiments revealed that the mutants

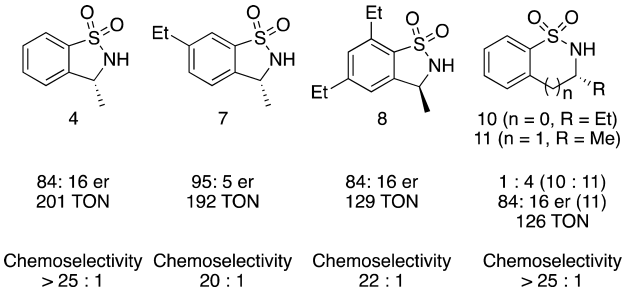

Figure 4. Outcome of C−H insertion reactions forming sultams **4**, **7**, **8**, and **11**. Conditions: 0.33 mol% Ir(Me)-PIX CYP119 (mutations C317G, T213G, V254L, F310G), 10 mM substrate, 0.5 mL solvent (100 mM Na-Pi, 100 mM NaCl, pH = 6.0 containing 2 vol% DMF), 37 °C, and 66 h. Chemoselectivity refers to molar ratio of sultam to sulfonamide products. Yield and TON refer to the formation of the sultam product. The absolute configuration of the products was determined by the experiments described in the Supporting Information.

which were most selective in the cell lysates were equally selective when used as purified enzymes. In particular, the mutant C317G, T213G, V254L, F310G catalyzed the formation of **4** with 84:16 er, 201 TON and 67% yield, in a reaction conducted at 37 °C with purified enzyme. The same mutant also catalyzed the formation of sultams **7**, **8**, and **11** in a highly enantioselective fashion, with up to 95:5 er, 192 TON and 64% yield. All reactions occurred with >20:1 chemoselectivity for C−H amination over reduction to the sulfonamide.

The reaction of 2-propyl benzenesulfonyl azide (**9**) can occur by insertion of the nitrene into the C−H bonds located at the benzylic or homobenzylic positions to form either a five- or a six-membered ring (**10** and **11**, respectively; Figure 4). The free Ir(Me)-PIX cofactor catalyzes the reaction of **9** to form a mixture of the five-membered product **10** and the six-membered product **11** with a slight preference for formation of **10** (60:40, **10**:**11**). On the other hand, the variant C317G, T213G, V254L, F310L of Ir(Me)-PIX CYP119 catalyzes the same reaction of **9** with opposite site selectivity, forming preferentially sultam **11** over sultam **10** (20:80, **10**:**11**), and with 84:16 er of product **11**, while producing less than 1% of the free sulfonamide byproduct. These results show that directed evolution can create Ir(Me)-PIX CYP119 catalysts for enantio-, chemo-, and site-selective C−H amination. Moreover, this study shows that evaluating variants of artificial metalloenzymes based on PIX-proteins containing abiological metals can be accomplished rapidly using cell lysates, without the need for protein purification or concentration.

To demonstrate further the potential of the Ir(Me)-PIX artificial metalloenzymes to catalyze C−H amination, we evaluated variants of Ir(Me)-PIX CYP119 as catalysts for the reaction of a sulfamate motif (Figure 5). The aryl sulfamates

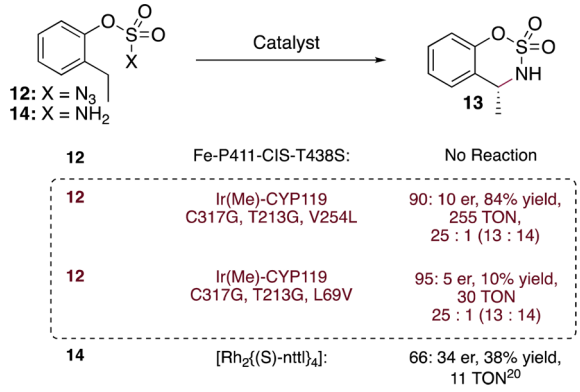

Figure 5. C−H amination of aryloxysulfonyl azides **12** to form aryl sulfamate **13** in the presence of Ir(Me) and Fe enzymes and a Rh catalyst. The conditions with Ir(Me)-PIX CYP119 mutants are the same as those in Figure 4. The conditions with Fe-P411-CIS mutant: 0.2% Fe-P411-CIS-T438S, 2 mM substrate, 2 mM $Na_2S_2O_4$, 1 mL solvent (100 mM KPi, pH 8.0 containing 2.5 vol% DMSO); only formation of sulfonamide **14** (50%) was observed. The results for the [Rh]-catalyzed reaction are given in ref 20. The absolute configuration of the product was determined by the experiments described in the Supporting Information.

formed by the amination reaction can undergo subsequent nickel-catalyzed cross-coupling reactions to form various chiral benzyl amines.[18,19] We found that the C−H amination of **12** does not occur in the presence of the variants of Fe-P450-BM3 that were reported previously for C−H amination reactions.[8] Moreover, the reaction of **12** in the presence of the free Ir(Me)-PIX cofactor formed only 7% of product **13** from nitrene insertion into the benzylic C−H bond. However, an evaluation of 20 mutants that generated active or selective enzymes for the reaction of substrate **2** revealed mutants that create more active catalysts. The variant C317G, T213G, V254L formed cyclized product **13** in 84% yield, 255 TON, 90:10 er, and >25:1 chemoselectivity for C−H bond amination over reduction to the sulfonamide. The reaction in the presence of the mutants C317G, T213G, L69V occurred to form **13** in even higher enantioselectivity (95:5 er), although the yield of the reaction was lower (10%). For comparison, the reaction of **14** catalyzed by chiral rhodium catalysts typically used for enantioselective C−H amination was reported to form product **13** in low yields and with low ee (up to 66:34 er).[20] By incorporating the abiological Ir(Me)-PIX cofactor into CYP119, we have created a catalyst that forms selectively a valuable class of molecules that has not been created previously with such selectivity by any natural enzymes or transition metal catalysts.

In summary, we have shown that Ir(Me)-PIX CYP119 enzymes catalyze C−H amination reactions with high chemoselectivity for insertion of nitrenes over reduction to the sulfonamide. Although Ir-containing porphyrins have not been reported to catalyze C−H amination reactions, Ir(Me)-PIX enzymes furnish sultams from sulfonyl azides in high yields,

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with high enantioselectivity and with good turnover numbers, while giving only traces of the sulfonamide byproducts typically observed in substantial amounts from the reactions catalyzed by Fe-PIX enzymes. Variants displaying these favorable selectivities were identified rapidly by screening mutants in cell lysates, instead of screening isolated, purified enzymes. Moreover, Ir(Me)-PIX CYP119 enzymes catalyze chemoselective C−H insertion reactions of aryloxysulfonyl azides that do not form any C−H amination products in the presence of natural enzymes and form the product with low yield and enantioselectivity with rhodium catalysts. Together, these results exemplify the merits of incorporating unnatural metals into PIX enzymes in order to achieve reaction outcomes previously not achieved using natural enzymes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b11410.

Experimental procedures, additional figures, tabulated experimental data, and complete characterization of new compounds reported in this publication (PDF)

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Author Contributions

⊥P.D. and H.M.K. contributed equally.

Notes

The authors declare the following competing financial interest(s): J.F.H., H.M.K., P.F.D., and D.S.C. are inventors on PCT Application No. PCT/US2016/057032, filed October 14, 2016, by the Lawrence Berkeley National Laboratory, that covers preparation and application of the artificial metalloenzymes containing iridium-porphyrins in this paper.

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