A “Broad Spectrum” Carbene Transferase for Synthesis of Chiral \( \alpha \)-Trifluoromethylated Organoborons

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Directed evolution generated an enzyme for the enantioselective synthesis of \( \alpha \)-trifluoromethylated organoborons—potentially attractive synthons for fluorinated compounds.

The realization that biocatalysis is one of the important elements for a more sustainable approach to chemical synthesis has resulted in a drive for finding enzymes for the catalysis of abiological reactions. The group of recent Nobel laureate Frances Arnold has pioneered the application of heme-containing enzymes for the catalysis of carbene transfer reactions. Key to this development was recognition that these reactions are mechanistically similar to the oxygen transfer reactions catalyzed by heme-containing enzymes such as cytochromes P450.\(^1,2\) This has led to a range of “carbene transferases”: engineered heme-containing enzymes for carbene transfer reactions such as cyclopropanations and X–H insertion reactions, including abiological Si–H and B–H insertions.\(^3,4\) In this issue of *ACS Central Science*, Houk, Arnold, and colleagues report an enzymatic platform for the enantioselective synthesis of a broad range of \( \alpha \)-trifluoromethylated (\( \alpha \)-CF\(_3\)\) organoborons, which are potentially attractive synthetic building blocks (synthons) for fluorinated compounds.\(^5\) The reaction involves the insertion of a carbene into the B–H bond of the N-heterocyclic carbene borane synthon 1 (Scheme 1), using diazotrifluoralkanes of general structure 2, as a carbene precursor.

The carbene transferases presented in this paper are derived from bacterial protein cytochrome c (denoted as *Rma cyt c*), which also served as the basis for the previously reported Si–H and B–H insertion enzymes. The enzyme was used in whole *Escherichia coli* cells where it was directed to the periplasm, and demonstrated better results in catalysis compared to the isolated proteins.\(^4\)

As expected, the wild-type protein showed no appreciable catalytic activity in the organofluorine synthesis. Therefore, site saturation mutagenesis of residues that could shape the binding pocket was undertaken, with a focus on residues that contribute to the binding of the carbene intermediate and borane substrate as well as residues in the surface loop (Figure 1). The rationale for this was that in previous work it was shown that mutations in this loop make it more dynamic.\(^4,6\) This approach was predicted to give rise to more open conformations and contribute to the formation of an active site where the CF\(_3\) group would be pointed into the heme pocket, and the bulkier alkyl substituent would be directed toward the solvent. The screening efforts resulted in the identification of the mutant enzyme, designated BOR-CF\(_3\), which gave rise to 2460 turnovers and an enantiomeric ratio of 97.5:2.5 in the reaction of 1 with 2a. Consistent with the hypothesis, BOR-CF\(_3\) indeed proved capable of converting a variety of diazoalkane substrates containing significantly different R groups, such as 2b, with 500–2900 turnovers and high enantioselectivities. A substrate with a smaller alkyl chain such as 2c was not converted.

The effect of the mutations and the origins of the stereocontrol was investigated by computational studies. To this aim, the substrate and the carbene intermediate were embedded into BOR-CF\(_3\) and subjected to large-scale molecular dynamics (MD) simulations. The results suggest that the CF\(_3\) group of the substrate and the carbene intermediate is nicely accommodated at the inner side of the cavity, due to the structural rearrangements promoted by a mutation. Additionally, residues located in the front loop and at the entrance of the cavity are suggested to stabilize the phenyl group of the substrate, which appears pointing toward the solvent. In this orientation, the pro-R face of the substrate is the only exposed toward the free volume of the cavity, which explains the observed preference for the R enantiomer. Furthermore, the enhanced dynamics of the front loop by mutations likely facilitates the entrance of the borane substrate 1, as well as generates an appropriate binding pocket.
pocket, to reach a catalytically competent configuration for the asymmetric B–C formation, as elucidated via quantum mechanical calculations. In agreement with the experimental results, docking and MD simulations show that the carbene intermediate derived from 2c containing a short-chain R group is not stabilized in near-attack-configurations.

Key to the activity of BOR-CF₃ are the mutations in the surface loop that covers the active site of the enzyme, which make the loop more dynamic and make the catalytic heme more accessible for the substrates. Notably, the combined mutations appear to have mainly a structural effect: the active site is "sculpted" such that it complements the CF₃ group on the carbene intermediate and provides a binding pocket for the borane substrate. On the other hand, the R group of the carbene intermediate is directed toward the solvent-exposed site. This means that the activity and the enantioselectivity in the C–B bond formation are predominantly controlled by the accurate positioning and restricted orientation of the CF₃ group, which all diazosubstrates have in common, and is independent of the structure of the R group, which differs in all the substrates. This is reflected in the broad scope of substrates that were converted with similarly high activity.

This work is yet another demonstration of the versatility of engineered heme enzymes for the catalysis of abiological reactions. Particularly elegant is the approach to achieve a broad scope by providing structural complementarity to structural elements that all substrates have in common, but making the variable part stick out toward the solvent, so the protein does not discriminate for this part of the structure. This approach may be applicable to many different enzyme design strategies as well. It would be interesting to see how the structure of BOR-CF₃ compares with those of the previously reported borylating enzymes BOR-P2 and BOR-G1,4 which do convert diazotrifluoralkane substrates with short alkyl or α-aryl side chains R,⁴,⁵ to understand how the different mutations in the same protein relate to this complementarity in substrate scope.

Finally, this study underscores the importance of considering structural dynamics in the design of enzymes for new-to-nature reactions, an aspect that is too often ignored, and the importance of MD simulations, in combination with other computation techniques, in accounting for these effects.⁶

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REFERENCES


