SITE-SELECTIVE CHEMICAL PROTEIN MODIFICATION

Reported by Antonio LaPorte October 29, 2019

INTRODUCTION

High reaction efficiency and selectivity are required of methods developed for small molecule synthetic organic chemistry. For the purposes of modifying proteins, among the most complex natural products, the same synthetic methods are used which thus presents new challenges. These chemical transformations must now accommodate a variety of new requirements including the preservation of protein function, high site-selectivity in the presence of an abundance of reactive moieties, and efficacy in aqueous media.[1] If successful, these methods can enable the study of post-translational modification effects, the study of protein dynamics and trafficking, and bioconjugation for the development of improved therapeutics.[2]

REACTIONS AT DEHYDROALANINE

Development of various strategies for the incorporation of dehydroalanine into proteins has inspired new chemical methods for the site-selective modification of this residue.[3] There are numerous examples of two-electron nucleophiles adding to electrophilic dehydroalanine residues, however, Davis and coworkers recently showed that carbon-centered radicals are also competent nucleophiles in these reactions. Addition of the C-centered radical, followed by quenching of the intermediate Cα radical, furnishes new Csp^3-Csp^3 connections (**Figure 1**).[5] This approach is viable in the incorporation of various natural and unnatural post-translational modifications as well as fluorinated and isotopically labeled side chains on numerous protein scaffolds.

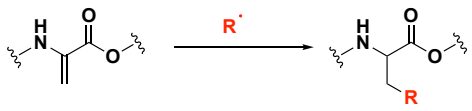

Figure 1. Selective modification of dehydroalanine residues with carbon-centered radicals.

PALLADIUM MEDIATED BIOCONJUGATION

The use of transition metals in the site-selective modification of proteins has been limited owing to necessarily strict reaction conditions and the abundance of Lewis basic functionality present in these macromolecules. Collaborative work performed by the Pentelute and Buchwald groups demonstrated that palladium oxidative addition adducts afford high selectivity for the arylation of cysteine residues (**Figure 2**).[6] These highly stable functional handles enabled the synthesis of novel stapled peptides and antibody-drug conjugates.

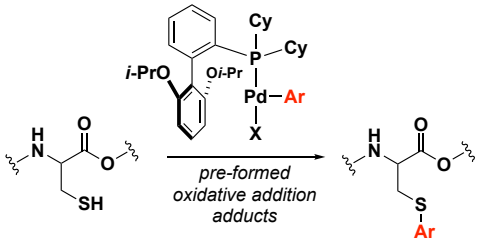

Figure 2. Oxidative addition adducts for the arylation of cysteine residues.

DECARBOXYLATIVE ALKYLATION

Photoredox catalyzed, radical-mediated decarboxylative alkylation has proved to be a practical tool for the synthesis of small molecules. Collaborative efforts between Bristol-Myers Squibb and the MacMillan Group translated this method to protein substrates (**Figure 3**).[7] A water compatible photocatalyst was developed enabling differences in oxidation potential to be exploited for the selective decarboxylative alkylation of C-terminal carboxylates in the presence of internal residues. This strategy is used in the C-terminal alkylation of insulin and illustrates that photoredox catalysis can be a viable method for site-selectively modifying proteins.

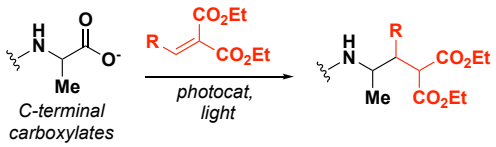

Figure 3. Bioconjugation through site-selective decarboxylative alkylation.

REACTIONS AT METHIONINE

Methionine has not been targeted extensively for chemical modification owing to its lower nucleophilicity relative to other residues such as cysteine and lysine. Modification of methionine could be advantageous because of its low abundance and limited function within proteins. Collaboration between the Toste and Chang research groups allowed development of new oxaziridine-based reagents that enable selective modification of methionine residues (**Figure 4a**).[8] This method is used to functionalize proteins, synthesize antibody-drug conjugates, and identify hyperreactive methionine residues. Gaunt and coworkers pioneered a similar approach in which hypervalent iodine reagents are used to install functional handles at methionine (**Figure 4b**).[9] These ethyl diazoacetate motifs are highly stable and can undergo functionalization through photoredox catalysis to furnish a variety of bioconjugates.

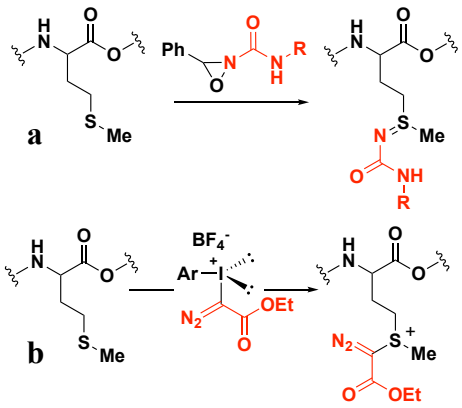

Figure 4. Bioconjugation enabled through site-selective reactions at methionine.

Collectively, these advances in site-selective chemical protein modification enable increased efficiency and generalizability when performing alterations required to gain insight into complex biological processes as well as designing new protein-based therapeutics.

References

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