ABSTRACT

This doctoral dissertation focuses on the use of enzymatic ligation to produce peptide bonds for the selective and functional semisynthesis and modification of proteins. It includes three interdisciplinary research projects united by a common goal: the development of selective, efficient, and mild methods for producing proteins and their conjugates. In the first project, a complete chemoenzymatic synthesis of metallothionein was demonstrated using the cyanobacterial protein SmtA by employing an engineered asparaginyl endopeptidase variant — OaAEP1_C247A. The developed strategy combined solid-phase peptide synthesis with enzyme-mediated ligation, leading to the production of a protein in high purity and yield. The final product displayed structural, spectroscopic, and functional properties identical to those of its recombinant counterpart, including the ability to specifically bind d-block metal ions (Zn^{2+}, Cd^{2+}) and proper native folding. These results provide evidence that the chemoenzymatic method is a viable alternative to recombinant expression for proteins with challenging, cysteine-rich sequences. The second project introduced a universal bifunctional probe, SrtCrAsH-EDT_2, enabling concurrent fluorescent labeling and installation of post-translational modifications (e.g., ubiquitination or sumoylation). This strategy is based on the combination of sortase A-catalyzed ligation with the specific binding of a biarsenical probe to tetracysteine tag within the protein backbone. Sequential one-pot reactions obviate the need for intermediate isolation, thus significantly simplifying and expediting the workflow. The approach exhibits high specificity and reversibility, as demonstrated in model experiments with GST-TC12 and HePTP-4C. This tool opens new avenues for probing the dynamics, localization, and function of post-translational modifications and holds promise for molecular diagnostics and live-cell imaging. In the third project, we engineered a novel butelase 1 variant, butelase AY, which exhibits markedly improved stability, a reduced aggregation propensity, and a simplified expression and activation protocol compared with the wild type enzyme. Butelase AY displays high catalytic efficiency and tolerates substrates containing both natural and non-natural amino acid residues, enabling effective peptide cyclization and fluorescent labeling of proteins. These attributes make it a powerful reagent for biomolecular modification under conditions requiring high specificity and mild reaction parameters. Consequently, these strategies furnish flexible, efficient, and selective platforms for the production and modification of proteins, especially for targets whose synthesis by conventional methods is challenging or unfeasible. The outcomes of this work have broad applicability across structural biology, molecular diagnostics, cellular imaging, and biomolecular engineering.