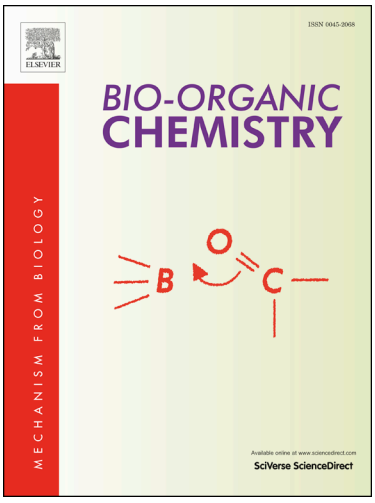

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Development of Optimized Conditions for Glaser-Hay Bioconjugations

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Abstract

The efficient preparation of protein bioconjugates represents a route to novel materials, diagnostics, and therapeutics. We previously reported a novel bioorthogonal Glaser-Hay reaction for the preparation of covalent linkages between proteins and a reaction parner; however, deleterious protein degradation was observed under extended reaction conditions. Herein, we describe the systematic optimization of the reaction to increase coupling efficiency and decrease protein degradation. Two optimized condtions were identified varying either the pH of the reaction or the bidentate ligand employed, allowing for more rapid conjugations and/or less protein oxidation.

Keywords: Bioconjugation; Glaser-Hay reaction; Alkyne; Unnatural amino acid

1. Introduction

With widespread applications in the fields of medicine, materials, and pharmaceuticals, bioconjugate chemistry is a rapidly growing area of chemical research. Bioconjugates are comprised of a biological macromolecule linked to a second molecule, often a surface, probe, nanoparticle, or another biomolecule.[1, 2] Protein bioconjugates, wherein at least one of the conjugate partners is a protein, have been utilized to enhance drug delivery and cellular imaging through the use of antibodies conjugated to cytotoxic drug molecules and luminescent quantum dots, in addition to numerous other applications.[3-9]

The preparation of covalently-linked protein bioconjugates is often accomplished through reaction of a protein's native nucleophilic residues, such as lysine, cysteine, and serine.[10] However, through this method, bioconjugation can occur at multiple residues within the protein, resulting in non-specific conjugation at a varying number of positions.[10, 11] To overcome this lack of selectivity, unnatural amino acids (UAAs) can be site-specifically introduced into proteins *via* suppression of the amber stop codon (TAG) by an evolved orthogonal amino acyl synthetase (aaRS)/tRNA pair.[12-14] The incorporation of a UAA bearing a chemical moiety not found within the twenty naturally occurring amino acids not only provides a specific site for conjugation of the protein, but also allows access to several useful conjugation methods previously unavailable for bioconjugation reactions involving proteins.[15, 16]