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Site-Specific Conjugation to Native and Engineered Lysines in Human Immunoglobulins by Microbial Transglutaminase

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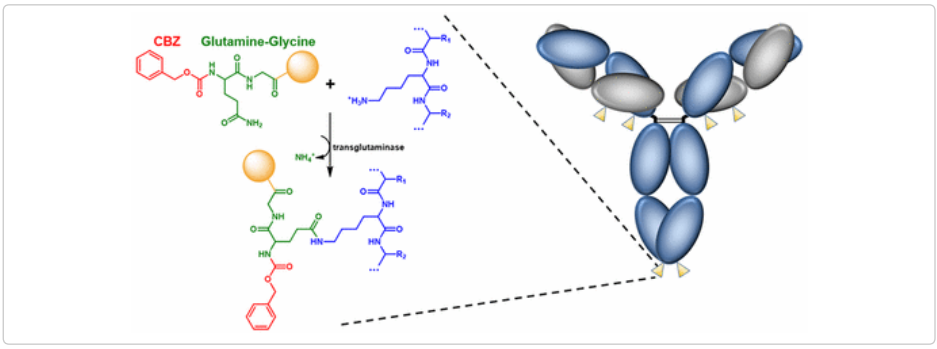
Abstract



The use of microbial transglutaminase (MTG) to produce site-specific antibody–drug conjugates (ADCs) has thus far focused on the transamidation of engineered acyl donor glutamine residues in an antibody based on the hypothesis that the lower specificity of MTG for acyl acceptor lysines may result in the transamidation of multiple native lysine residues, thereby yielding heterogeneous products. We investigated the utilization of native IgG lysines as acyl acceptor sites for glutamine-based acyl donor substrates. Of the approximately 80 lysines in multiple recombinant IgG monoclonal antibodies (mAbs), none were transamidated. Because recombinant mAbs lack the C-terminal Lys447 due to cleavage by carboxypeptidase B in the production cell host, we explored whether blocking the cleavage of Lys447 by the addition of a C-terminal amino acid could result in transamidation of Lys447 by a variety of acyl donor substrates. MTG efficiently transamidated Lys447 in the presence of any nonacidic, nonproline amino acid residue at position 448. Lysine scanning mutagenesis throughout the antibody further revealed several transamidation sites in both the heavy- and light-chain constant regions. Additionally, scanning mutagenesis of the hinge region in a Fab′ fragment revealed sites of transamidation that were not reactive in the context of the full-length mAb. Here, we demonstrate the utility of single lysine substitutions and the C-terminal Lys447 for engineering efficient acyl acceptor sites suitable for site-specific conjugation to a range of glutamine-based acyl donor substrates.

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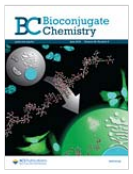

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