ENGINEERING SUBSTRATES OF TRANSGLUTAMINASE USING THE GLUTAMINE-WALK STRATEGY FOR SPECIFIC MODIFICATION OF IGG1 ANTIBODIES

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Microbial transglutaminase (mTG) is a robust enzyme used on a large scale in the food industry. Its well-established safety and efficacy make it increasingly popular for biopharmaceutical engineering applications. MTG efficiently catalyzes protein cross-linking by forming stable amide bonds between a glutamine and a lysine. Instead of lysine, the enzyme can be diverted to graft amine-bearing substrates onto proteins, expanding its utility into the realm of protein labelling. Since many proteins of interest lack native reactivity with mTG, we introduce the glutamine-walk, a straightforward method to create a mTG-reactive glutamine-substrate site in a protein of interest.

To that effect, we report engineering an antibody to become an effective substrate of mTG. Antibody-drug conjugates are composed of a monoclonal antibody bound to a cytotoxic drug, combining the efficacy of chemotherapy with the high specificity of the antibody for the treatment of cancer. MTG allows the conjugation of aminated compounds onto a glutamine (Q295) of the human crystallizable fragment (hFc) of deglycosylated antibodies. However, the deglycosylation process reduces antibody stability and solubility, limiting its use for industrial-scale preparation. We individually substituted surface-exposed residues of IgG1 hFc to glutamines. Reactivity of mTG towards the new variants was determined by conjugation to aminated fluorophores. The most reactive variant was validated in the context of a complete antibody, trastuzumab, used for the treatment of Her2+ breast cancer. Conjugated with the cytotoxic monomethylauristatin E confirmed that antigen binding and compound cytotoxicity were maintained. These results are promising for the use of mTG in the synthesis of antibody-drug conjugates. The approach is empirical and will enable selective labelling of proteins that are otherwise unreactive with mTG.