Transglutaminase Enzymatic site-selective PEGylation of proteins at lysine or glutamine

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INTRODUCTION

Enzymatic approaches of protein PEGylation are widening their applications thanks to the advantageous obtainment of homogeneous mono-PEGylated isomers. Microbial transglutaminase (mTGase) is an enzyme that, in nature, forms protein cross-linkings between side chains of Gln and Lys residues. For protein PEGylation, mTGase allows site-specific modification with PEG-NH_2 at precise glutamines of the proteins. We have already reported mTGase-mediated conjugation with PEG-NH_2 to G-CSF, yielding a site selective mono-derivative conjugate involving Gln135 [1]. Interestingly, the same enzymatic reaction of mTGase, was performed using a new PEG derivative, PEG (PEG-Z-QG), containing a Gln substrate of mTGase, in order to target the Lys residues and investigate if the selectivity of the enzyme is maintained also in this reverse conjugation method. The obtained conjugate was then compared to PEG-Q135-G-CSF isomer, in terms of biophysical characterization and pharmacokinetics [2]. In another work, an immobilized form of TGase has been studied with the aim to use it as an alternative tool for protein PEGylation that presents the advantage of a simply removal of mTGase from reaction mixture by centrifugation. The immobilized mTGase (iTGase) was characterized in term of retained activity, kinetic parameters and in different pH and temperature values. For protein PEGylation, a site-specific modification of G-CSF and α-lactalbumin (α-LA), comparing the two forms of mTGase, was then tested [3].

RESULTS AND FUTURE PERSPECTIVES

The mTGase-mediated conjugation of PEG-ZQG in the presence of G-CSF generated a PEG-G-CSF conjugate in which the polymer was selectively coupled to Lys41 of the protein. The PEG-K41-G-CSF conjugate was formed in high yield and the mTGase maintained high selectivity also for the lysine modification sites. PEG-K41-G-CSF was compared to PEG-Q135-G-CSF in biophysical studies, demonstrating that the two positional isomers have similar behaviors, while the pharmacokinetics in rats have exhibited comparable half-life extensions. mTGase has been demonstrated to be a valuable tool for double site-selective protein modification, either to Gln or Lys residues and can thus offer relevant opportunities in the field of selective protein derivatization, providing, at the same time two potential sites of conjugation, both with high selectivity. In the second purposed strategy, mTGase was covalently immobilized on a beaded agarose resin. The iTGase preserved more than 53% of its starting activity and revealed, as expected, a reduction of the affinity for the substrate, that is, nevertheless, counterbalanced by the increasing in the stability against different pH and temperature conditions. iTGase-mediated PEGylation of α-LA showed a selective conjugation toward only one Gln residue of α-LA, when using iTGase, avoiding the formation of a mono and bi-conjugate mixture, achieved using the free enzyme. In the case of G-CSF, iTGase still remained selective towards only one Gln, forming PEG-Q135-G-CSF, but avoided the undesired formation of deamidated G-CSF that took place when free mTGase was used. In conclusion, iTGase has shown an increase in thermal stability and substrate selectivity, suggesting that the immobilization strategy could positively modify the conjugation outcomes and yield pure mono-PEGylated-protein conjugates. Overall, these results highlight the potentiality of mTGase in preparing site-selective protein-polymer conjugates and it would be interesting to test the applicability of these new approaches also to other therapeutic proteins.

References:

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