Enzymatic synthesis of γ-glutamyl derivatives catalyzed by a new mutant γ-glutamyltransferase with improved transpeptidase activity

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Despite their potential applicative interest as biologically active compounds and as flavor enhancers, γ-glutamyl derivatives are commercially underexploited compounds. This is mainly due to the difficulties connected with their supply at a reasonable cost. As a consequence, enzymatic approaches to their preparation, based on the use of γ-glutamyltransferases (GGTs), have been proposed[1] to circumvent both the low-yielding extractive procedures from natural sources and the troublesome chemical synthesis, rendered uneconomical by the need of protection and deprotection steps.

GGTs catalyze the transfer of a γ-glutamyl moiety from a donor substrate (e.g. glutathione) to the primary amino group of an acceptor compound in a so-called transpeptidation reaction, through the formation of a γ-glutamyl-enzyme intermediate. However, also the use of GGTs as biocatalysts is not free from drawbacks. In addition to the transpeptidase activity, GGTs show a non-negligible hydrolase activity towards both the donor substrate and the newly formed transpeptidation product, affording irreversibly glutamic acid.[2]

In our ongoing studies on bacterial GGTs, we found that the presence of the lid loop – a short amino acids sequence covering the active site in most of the known GGTs – not only affects substrate selection, but also modulates hydrolase/transpeptidase activities.[3] Within the TailGluTran Project,[4] aimed at the development of mutant GGTs with improved transpeptidase activity, is currently under investigation a mutant enzyme obtained by inserting the sequence of the lid loop on the structure of a GGT naturally lacking it. The mutant enzyme shows promising high transpeptidase activity with respect to wild type counterparts and represents a starting point for further modifications in the search of a suitable biocatalyst intended for preparative purposes.

References:

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[3] C. Calvio, F. Romagnuolo, F. Vulcano, G. Speranza, C. F. Morelli, *Enz. Micr. Technol*. **2018**, *114*, 55-62
[4] The TailGluTran Project is founded by Fondazione Cariplo, Bando 2016 sulle Bietecnologie Industriali e la Bioeconomia, n. 2016-0741.