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

Michela Massone,[a] Cinzia Calvio,[b] Giovanna Speranza,[a] Carlo F. Morelli[a]

[a]*Università degli Studi di Milano, Dipartimento di Chimica via Golgi, 19 -20133 Milano*

[b]*Università degli Studi di Pavia Dipartimento di Biologia e Biotecnologie "Lazzaro Spallanzani" via Ferrata, 9 – 27100 Pavia*

e-mail: *carlo.morelli@unimi.it*

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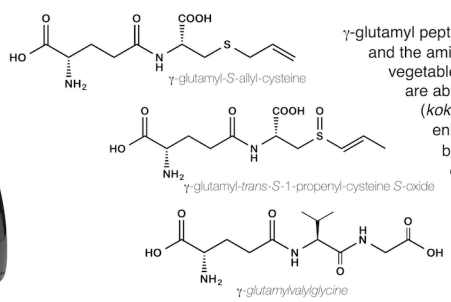
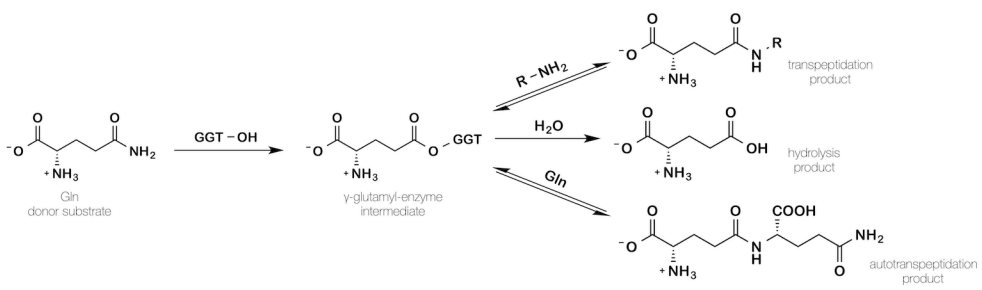


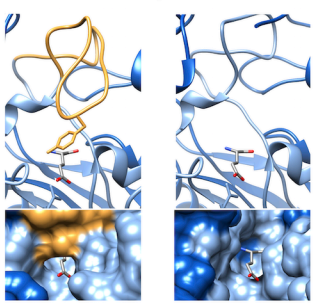
γ-glutamyl peptides are compounds characterized by an amide bond involving the γ-carboxyl group of a glutamic acid residue and the amino group of an amino acid or a short peptide. They are naturally occurring substances found in several edible vegetables and synthesized by bacteria during food fermentation and aging. They are nearly tasteless by themselves, but are able to elicit strong flavor-enhancer activity increasing mouthfulness, balance and long-lasting savory sensations (*kokumi* properites), mostly in the presence of protein-rich food. γ-glutamyl-*S*-allyl-cysteine is a *kokumi* flavor enhancer of garlic;[1] γ-glutamyl-*trans*-*S*-1-propenyl-cysteine *S*-oxide has interesting biological activity in relation to bones resorption phenomena[2] and γ-glutamylvalylglycine from soy sauce is among the most potent *kokumi* compounds known.[3]

Despite their applicative and economical potential, γ-glutamyl derivatives are commercially underexploited compounds, due to the difficulties connected with their supply at a large scale at reasonable costs. Extraction from natural sources is laborious, low-yielding and erratic, due to the natural variability of the vegetable raw materials. Synthesis through classical peptide chemistry is rendered not economical by the need of protection and deprotection steps. In this scenario, enzymatic approaches based on the use of γ-glutamyltransferases (GGTs) of different origin could offer a viable alternative.[4]

GGTs are widespread enzymes able to catalyze the transfer of a γ-glutamyl moiety from a donor substrate (glutathione, glutamine) to the primary amino group of an acceptor compound through a γ-glutamyl-enzyme intermediate. However the use of GGTs as biocatalysts for preparative purposes involves some problems.
The γ-glutamyl-enzyme intermediate can be irreversibly hydrolyzed by attack of a water molecule leading to glutamic acid. The compound used as the donor substrate, e.g. glutamine, can act also as the acceptor one in an autotranspeptidation reaction affording γ-glutamylglutamine. Finally, the reaction product is still a substrate able to react as a γ-glutamyl donor.



The mutant enzyme LL-Bsub GGT



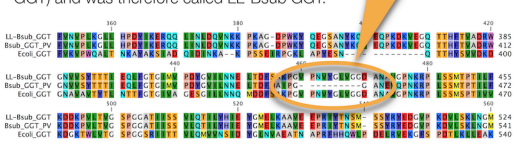
Active site of lid+ GGT (*E. coli*) with bound glutamate

Active site of lid– GGT (*B. subtilis*) with bound glutamate

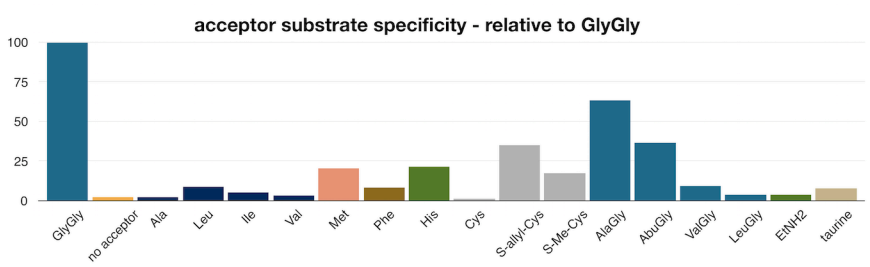
In our ongoing studies on bacterial GGTs,[5] we found that the presence of a short amino acid sequence called lid-loop, covering the active site of most GGTs, is able not only to affect substrate selection, but also to modulate hydrolase/ transpeptidase activities.[6]

inserted lid-loop

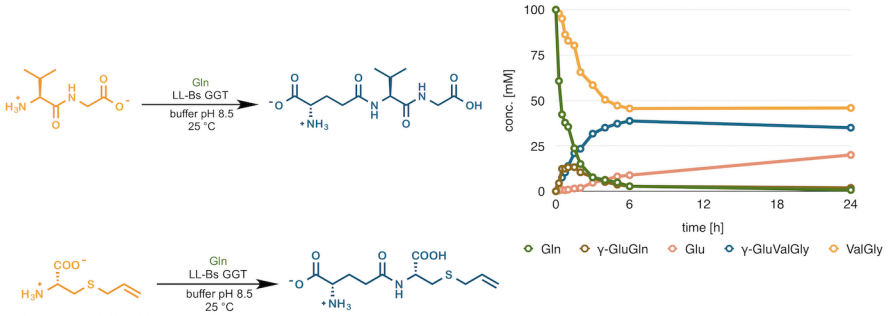
The mutant enzyme was constructed by inserting the sequence of the lid-loop of *E. coli* GGT (Ecoli GGT) into the structure of *B. subtilis* GGT (Bsub GGT) and was therefore called LL-Bsub GGT.

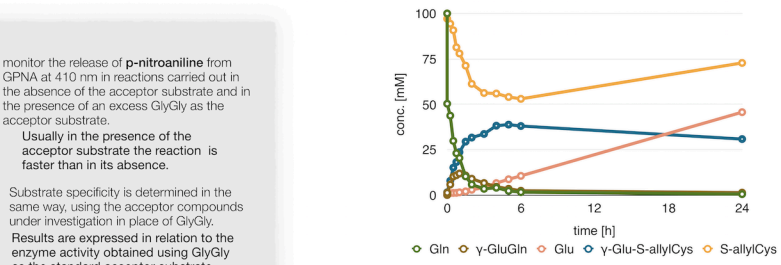


LL-Bsub GGT characterization

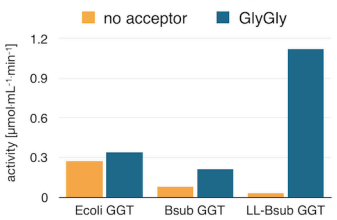


First examples of synthetic applications - unoptimized conditions

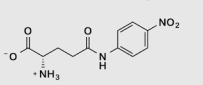




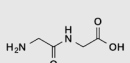
improved transpeptidase activity - standard assay -



the standard GGT assay in brief



GPNA, chromogenic donor



GlyGly, standard acceptor

monitor the release of **p-nitroaniline** from GPNA at 410 nm in reactions carried out in the absence of the acceptor substrate and in the presence of an excess GlyGly as the acceptor substrate.

Usually in the presence of the acceptor substrate the reaction is faster than in its absence.

Substrate specificity is determined in the same way, using the acceptor compounds under investigation in place of GlyGly.

Results are expressed in relation to the enzyme activity obtained using GlyGly as the standard acceptor substrate.

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