A MUTANT γ-GLUTAMYLTRANSFERASE WITH IMPROVED TRANSEPTIDASE ACTIVITY

Michela Massone1, Cinzia Calvio2, Alessandra M. Albertini2, Giovanna Speranza1, Carlo F. Morelli2

1Università degli Studi di Milano Dipartimento di Chimica, via Golgi, 19 - 20133 Milano
2Università degli Studi di Pavia Dipartimento di Biologia e Biotecnologie “Lazzaro Spallanzani”, via Ferrata, 9 - 27100 Pavia
michela.massone@unimi.it

γ-Glutamyl derivatives of both unmodified and modified amino acids are naturally occurring flavor enhancers found in many foods such as cheese, garlic, mushrooms and fermented sauces, as soy sauce, used in cuisine worldwide. Despite their potential applicative, they are commercially underexploited compounds due to the difficulties connected with their supply at a reasonable cost. Enzymatic approaches to their preparation based on the use of γ-glutamyltransferases (GGTs) as biocatalysts have been proposed to circumvent both the low-yielding extractive procedures from natural sources and the uneconomical chemical synthesis. However, also the use of GGTs is not free from drawbacks because of the hydrolase activity towards both the donor substrate and the newly formed transpeptidation product, affording irreversibly glutamic acid.

AIM and RESULTS

Development of mutant GGTs with improved transpeptidase activity, to be used as biocatalysts in the synthesis of γ-glutamyl derivatives of commercial interest.

Sequence alignment of B. subtilis - LL GGT, B. subtilis GGT and E. coli GGT, showing the insertion of the «Loop Loop» sequence which covers the E. coli GGT’s binding site, into the structure of B. subtilis GGT, which lacks it, to give the mutant B. subtilis - LL GGT.

Comparison between activities (μmol s⁻¹ ml⁻¹) of B. subtilis GGT, E. coli GGT and B. subtilis - LL GGT in the absence and presence of glycylglycine as acceptor, at two different pHs.

Mutant B. subtilis - LL GGT shows an improved transpeptidase activity at a pH close to neutrality.

Ratio between transpeptidase and hydrolase activities of B. subtilis GGT, E. coli GGT and B. subtilis - LL GGT at two different pHs.

Substrate specificity of mutant B. subtilis - LL GGT towards different acceptors at pH 8.5.

1Shao, C.; Scheller, A.; Garant, M.; Food Res. Int. 2010, 43, 39-47.
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