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γ-Glutamyl compounds and their enzymatic production using bacterial γ-glutamyltranspeptidase

Review Article

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Summary. Some amino acids and peptides, which have low solubility in water, become much more soluble following γ-glutamylation. Compounds become more stable in the blood stream with γ-glutamylation. Several γ-glutamyl compounds are known to have favorable physiological effects on mammals. γ-Glutamylation can improve taste and can stabilize glutamine in aqueous solution. Because of such favorable features, γ-glutamyl compounds are very attractive. However, only a small number of γ-glutamyl amino acids have been studied although many other γ-glutamyl compounds may have characteristics that will benefit humans. This is mainly because γ-glutamyl compounds have not been readily available. An efficient and simple method of producing various γ-glutamyl compounds, especially γ-glutamyl amino acids, using bacterial γ-glutamyltranspeptidase has been developed. With this method, modifications of reactive groups of the substrate and energy source such as ATP are not required, and a wide-range of γ-glutamyl compounds can be synthesized. Moreover, bacterial γ-glutamyltranspeptidase, a catalyst for this method, is readily available from the strain over-producing this enzyme. The superiority of producing γ-glutamyl compounds with bacterial γ-glutamyltranspeptidase over other methods of production is discussed.

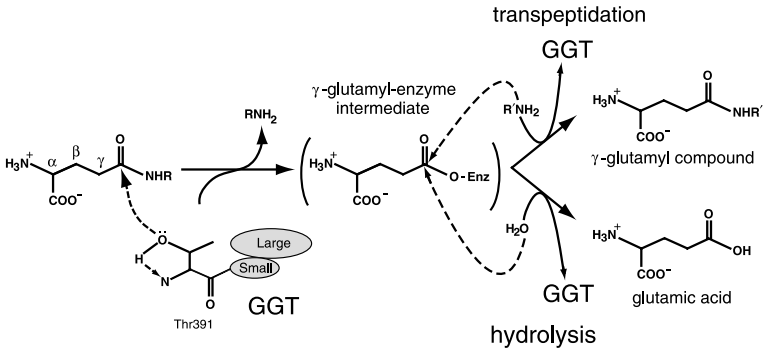
Keywords: Amino acid – γ-Glutamyl transferase – γ-Glutamyl compounds – γ-Glutamylation – Transpeptidation – Enzymatic production

Introduction

An amide linkage between the γ-carboxyl group of glutamic acid and amino group of some compound is called a γ-glutamyl linkage. When the C-terminal is an amino acid, the linkage is a γ-peptide linkage. γ-Glutamyl compounds are not scarce in nature. Glutathione (γ-glutamylcysteinylglycine) is the most abundant free thiol compound in cells (Meister and Anderson, 1983). Glutamine is the most abundant free amino acid in living organisms (Curi et al., 2005). The bacterial cell wall consists of peptidoglycan which has a γ-glutamyl linkage (Park, 1996). Moreover, some bacteria have capsules made of poly-γ-glutamic acid and the relation of these capsules to virulence is well known in *Bacillus anthracis* (Makino et al., 1989). Folic acid usually exists as γ-glutamyl derivatives (Green et al., 1996). Some γ-glutamyl compounds have been reported to exist in the mammalian brain albeit in very small quantities (Yamamoto et al., 1992; Bittner et al., 2005).

γ-Glutamyltranspeptidase (GGT; EC 2.3.2.2), a heterodimeric enzyme, is found from bacteria to mammals and is involved in the metabolism of glutathione (Taniguchi and Ikeda, 1998). It catalyzes the cleavage of the γ-glutamyl linkage of γ-glutamyl compounds, such as glutathione, and the transfer of their γ-glutamyl moiety to other amino acids and peptides (Scheme 1). The activated oxygen atom of the side chain of the N-terminal threonine residue of the small subunit attacks the carbonyl carbon of γ-glutamyl compounds to form a γ-glutamyl-enzyme intermediate (Inoue et al., 2000). If this intermediate undergoes nucleophilic substitution by amino acids or peptides, it is a transpeptidation reaction forming new γ-glutamyl compounds. Employing various γ-glutamyl acceptors, one can synthesize various γ-glutamyl compounds using GGT. If the intermediate undergoes a nucleophilic attack by water, it is a hydrolysis reaction releasing glutamic acid. The pH optima of these two reactions are different. Therefore, by adjusting the pH of the reaction mixture, it is possible to make the enzyme catalyze one of the reactions selectively.

In this review, the distinct chemical and physiological characteristics of γ-glutamyl compounds will be described



Scheme 1. Reaction mechanism of GGT

and the advantages of the enzymatic production of γ-glutamyl compounds using bacterial GGT will be discussed.

Characteristics of γ-glutamyl compounds

Increase in solubility with γ-glutamylation

When some compounds are γ-glutamylated, their characteristics become very different. Some amino acids and peptides which have low solubility in water become much more soluble following γ-glutamylation. For example, the solubility of cystine in water is very low. When cystine was γ-glutamylated, its solubility was dramatically increased; by more than three orders of magnitude (Hara et al., 1992). Although the solubility of alanylcystine was very high compared with that of cystine, it was less soluble than γ-glutamylcystine.

Solubility in water is critical to the use of a compound for medication. A compound, which cannot be administered orally, has to be taken through either injection or an intravenous drip. A medication with low solubility has to be taken intravenously with a large volume of fluid and this is a major burden for debilitated patients.

Increase of stability in serum by γ-glutamylation

Another feature of γ-glutamyl compounds is that they are more stable in blood stream. Since the γ-glutamyl linkage cannot be cleaved by normal peptidases in serum, the half lives of compounds become much longer with γ-glutamylation (Hara et al., 1992) and the γ-glutamyl linkage is first cleaved in organs which express GGT. Therefore, some γ-glutamyl compounds may possibly be used as pro-drugs specific for the organs that express GGT. It was shown that γ-glutamyl-dermorphin could be used as a pro-drug with antinociceptive effects (Misicka et al., 1996). Another example is that the concentration of dopamine markedly increased not only in kidney but also in brain when γ-L-glutamyl-L-dihydroxyphenylalanine (DOPA) was administered to animals (Wilk et al., 1978; Ichinose et al., 1987). This indicates that γ-L-glutamyl-L-DOPA is promising as a pro-drug for Parkinson's disease.

Various physiological effects of γ-glutamyl compounds

Several γ-glutamyl compounds have been reported to have various physiological effects on mammals. For example, some γ-D-glutamyl compounds, such as γ-D-glutamylaminomethylphosphonate, γ-D-glutamylglycine, γ-D-glutamyltaurine, and γ-D-glutamyl-β-alanine, have antagonistic effect on the excitatory nervous system (Davies et al., 1982). An anticonflict effect (Kuribara and Tadokoro, 1982), and a potent and long-lasting antiepileptic action (Uemura et al., 1992) are known for γ-L-glutamyltaurine. Its effects on the metamorphosis of amphibians (Feuer et al., 1978, 1979), and on the concentration and activity of plasma renin (Feuer and Gaal, 1979), a radiation-protective effect (Feuer and Benko, 1981), and a positive ionotropic effect on the locust heart (Feuer and S-Rozsa, 1981) have all been reported. There has been an extensive recent review for γ-L-glutamyltaurine (Bittner et al., 2005). L-Theanine (γ-L-glutamylethylamide) decreases the blood pressure of spontaneously hypertensive rats (Yokogoshi et al., 1995), and inhibits convulsive action (Kimura and Murata, 1971) and the excitation caused by caffeine (Kimura et al., 1975). It has been shown that orally administered theanine is absorbed into the blood stream through the intestinal tract (Kitaoka et al., 1996; Unno et al., 1999). Theanine is also known to be incorporated into the brain through the blood-brain barrier *via* a Leu-preferring transport system, and may affect the metabolism and/or release of some neurotransmitters in the brain (Yokogoshi et al., 1998). It seems that many γ-glutamyl compounds have a tranquilizing effect on the excited nervous system and might be useful for stressful modern lives. More γ-glutamyl compounds should be

screened and an efficient method of producing γ-glutamyl compounds is essential.

Improvement of taste on γ-glutamylation

Another favorable feature of γ-glutamyl compounds is that some, such as theanine, taste good. Theanine is not only the main free amino acid component of tea leaves, but also the major umami component of tea (Sakato, 1949). There is a positive correlation between the grade of Japanese green tea and the amount of theanine in the tea (Goto et al., 1996).

γ-Glutamylation can improve the taste of bitter amino acids. In Japan, amino acids are added to nutritional supplements at high concentrations. The major types added are branched-chain amino acids, aromatic amino acids, and basic amino acids, some of which are essential amino acids. However, these L-amino acids taste bitter and this is a crucial problem to take these amino acids orally. It was found that when a bitter amino acid is γ-glutamylated, its bitterness is reduced dramatically and the sourness is increased (Suzuki et al., 2002a). This sourness, however, is a refreshing lemon-like taste, and so the preference for the γ-glutamyl amino acid was increased (Fig. 1). Since bacterial GGTs prefer aromatic amino acids and basic amino acids (Suzuki et al., 1986a; Minami et al., 2003) as a γ-glutamyl acceptor, it is easy to γ-glutamylate these amino acids using bacterial GGT. In contrast, branched-chain amino acids are poor substrates. The method has been improved and γ-glutamyl branched-chain amino acids can be produced with fairly good efficiency (Suzuki et al., 2004a).

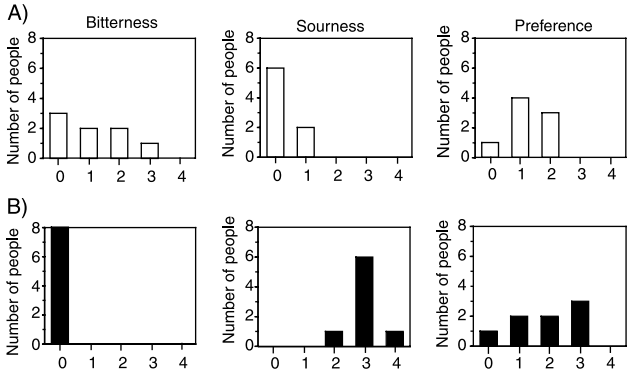


Fig. 1. Comparison of the tastes of phenylalanine (**A**) and γ-glutamylphenylalanine (**B**). The vertical axes indicate numbers of people. The horizontal axes for bitterness and sourness indicate the intensity of the taste: *0* did not feel; *1* felt slightly; *2* felt weakly; *3* felt; *4* felt strongly. The horizontal axes for preference indicate preference on a 5-point scale where 0 is dislike, 2 is neither, and 4 is like. The concentrations of phenylalanine and γ-glutamylphenylalanine used were 15 mM

Increase in stability of glutamine by γ-glutamylation

Glutamine protects the intestinal tract (Thomas et al., 2005) and liver (Bergamini et al., 1995), and increases immune competence (Newsholme and Parry-Billings, 1990). It was also reported that the degradation of protein from muscle by exercise was decreased by the ingestion of glutamine (Newsholme and Parry-Billings, 1990). Therefore, glutamine is not only used in the medical field but is also added to some supplements on the market. The problem with using glutamine in these fields is that glutamine in aqueous solution is unstable and readily converted to pyroglutamic acid. When 1 mM glutamine and γ-glutamylglutamine solutions (pH 7) were boiled, about 20% of the glutamine was lost after 30 min while practically no γ-glutamylglutamine was lost. When 1 mM glutamine, γ-glutamylglutamine, and alanylglutamine solutions (pH 7) were autoclaved for 20 min, about 97% of the glutamine and 20% of the γ-glutamylglutamine were lost while practically no alanylglutamine was lost (Yamada and Suzuki, unpublished data). The result indicates that γ-glutamylation can stabilize glutamine in aqueous solutions, but alanylglutamine is more stable than γ-glutamylglutamine. One major advantage of γ-glutamylglutamine over alanyl-

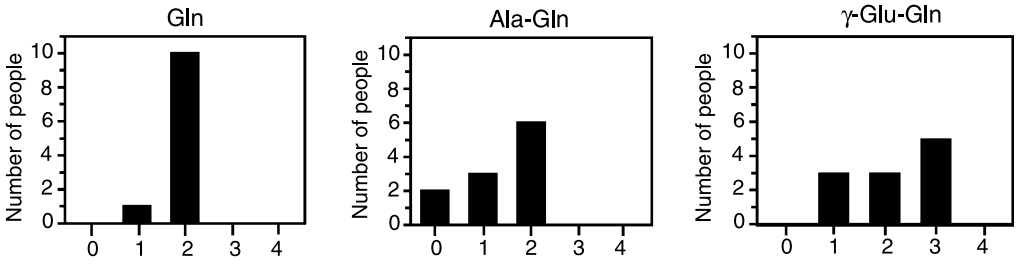


Fig. 2. Comparison of the tastes of glutamine, alanylglutamine and γ-glutamylglutamine. The vertical axes indicate numbers of people. The horizontal axes indicate preference on a 5-point scale where 0 is dislike, 2 is neither, and 4 is like. The concentrations of glutamine, alanylglutamine and γ-glutamylglutamine used were 10 mM

glutamine is its taste. Glutamine tastes better than alanylglutamine, and γ-glutamylglutamine tastes much better than glutamine (Fig. 2). This is a very important point when a glutamine derivative is taken orally.

Because of the features described above, γ-glutamyl compounds are very attractive.

Standard method to produce γ-glutamyl compounds using bacterial GGT

Glutamine as a γ-glutamyl donor and a compound with an amino group such as an amino acid as a γ-glutamyl acceptor are dissolved in water, and the pH of the mixture is adjusted to the optimum. A buffer, such as phosphate buffer, is not used because amino acids are used at high concentrations and they have buffering ability themselves. Then, bacterial GGT is added and the reaction mixture is incubated at 37 °C. The reaction conditions should be optimized for each γ-glutamyl compound. Usually the yield of the γ-glutamyl compound depends on the pH of the reaction mixture, the concentration and ratio of γ-glutamyl donor/acceptor, and the concentration of GGT. The optimum pH of the reaction mixture mainly depends on the *pKa* value of the amino group of the acceptor. When a poor γ-glutamyl acceptor to which the transfer of γ-glutamyl moiety is difficult is used, the yield is often improved by increasing the ratio of γ-glutamyl acceptor to donor.

The optimum reaction conditions and yields of various γ-glutamyl compounds for the enzymatic method using bacterial GGT are summarized in Table 1.

Examples of γ-glutamyl compounds produced by the enzymatic method using bacterial GGT

Examples of γ-glutamyl compounds which have been shown to be produced effectively by bacterial GGT are described below.

γ-L-Glutamyl-L-DOPA

γ-L-Glutamyl-L-DOPA was produced in high yield by bacterial GGT (Kumagai et al., 1988). Since DOPA is readily oxidized, ascorbic acid was added to the reaction mixture as an antioxidant. After the administration of γ-L-glutamyl-L-DOPA synthesized by bacterial GGT to mice, the amount of dopamine in the brain increased markedly (Ichinose et al., 1987). Hence, γ-L-glutamyl-L-DOPA as a pro-drug for treating Parkinson's disease is promising. γ-L-Glutamyl-L-DOPA was also shown to be a suitable substrate for sensitive electrochemical measurements of the enzymatic activity of human serum GGT, which is a marker of hepatomas and alcoholic hepatic diseases in blood tests (Kiuchi et al., 1986).

γ-Glutamyltaurine

γ-L-Glutamyltaurine has various physiological effects on mammals as mentioned above. During the development of an enzymatic method for the production of various γ-glutamyl compounds, it was found that the yield of a γ-glutamyl compound with a γ-glutamyl acceptor, such as taurine, was not very high. Not only γ-glutamylglutamine,

Table 1. The optimum reaction conditions and yields of various γ-glutamyl compounds

γ-Glutamyl compound	Concentration			pH	Yield	
	L-Gln (mM)	Acceptor (mM)	GGT (mU/ml)		(%)	(g/L)
γ-L-Glu-L-DOPA	200	200	250	10.6	79	51.5
γ-L-Glu-L-His	300	300	200	9.7	48	41.2
γ-L-Glu-L-Tyr methylester	300	300	200	7.3	37	35.7
γ-L-Glu-L-Phe	200	200	500	10.4	70	41.2
γ-L-Glu-L-Val	20	300	40	10.0	88[a]	4.3
S-Benzyl-glutathione monoethylester	200	100	200	6.2	76[b]	31.2
L-Theanine (γ-L-Glu-ethylamide)	200	1500	400	10.0	61[a]	21.1
γ-L-Glu-taurine	200	200	200	10.0	25	12.7
D-Theanine (γ-D-Glu-ethylamide)	200	1500	400	10.0	74[a]	25.6
γ-D-Glu-taurine	200	200	200	10.0	71	36.1
γ-D-Glu-L-Trp	50	50	200	9.0	66	11.0

[a] The yield against the donor
[b] The yield against the acceptor

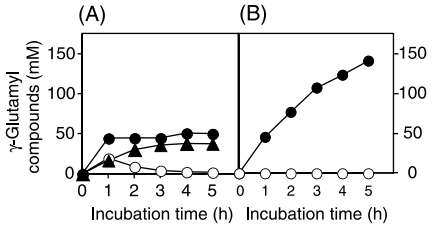


Fig. 3. Enzymatic synthesis of γ-glutamyltaurine with L-glutamine (**A**) and D-glutamine (**B**), as a γ-glutamyl donor. The reaction mixture contained 200 mM glutamine, 200 mM taurine, and 0.2 U/ml GGT, at pH 10 and 37 °C. Symbols: γ-glutamyltaurine (●), γ-glutamyl-γ-glutamyltaurine (▲), and γ-glutamylglutamine (○)

but also γ-glutamyl-γ-glutamyltaurine in a non-negligible amount, was formed (Suzuki et al., 2002b). This is mainly because GGT prefers glutamine and γ-glutamyltaurine as better γ-glutamyl acceptors than taurine. However, the yield was improved by increasing the ratio of γ-glutamyl acceptor to donor, that is, taurine to glutamine.

GGT can utilize D-glutamine as a γ-glutamyl donor, but it cannot utilize D-amino acids as a γ-glutamyl acceptor. Therefore, γ-D-glutamyl-D-glutamine and γ-D-glutamyl-γ-D-glutamyltaurine should not be synthesized as by-products when D-glutamine is used as a γ-glutamyl donor. The *Km* value for D-glutamine is 278 μM and is much larger than that for L-glutamine, 8.3 μM. However, both values are small enough for the enzymatic production of γ-glutamyl compounds in which several hundreds mM of glutamine is used (Suzuki et al., 2003). When D-glutamine was used instead of L-glutamine, the yield of γ-glutamyltaurine was dramatically improved (Fig. 3). The purified product was identified with a polarimeter as γ-D-glutamyltaurine which also has a preferable physiological effect (Suzuki et al., 2003; Davies et al., 1982).

γ-D-Glutamyl-L-tryptophan

γ-D-Glutamyl-L-tryptophan stimulates the differentiation of T-lymphocyte and specific immune response, and enhances interleukin 2 and interferon γ production in mice (Simbirtsev et al., 2003). In phase 2 clinical trials, γ-D-glutamyl-L-tryptophan in combination with standard chemotherapy was very effective in the treatment of tuberculosis (Orellana, 2002). Also its oral administration may be effective. Therefore, γ-D-glutamyl-L-tryptophan is a prospective medicine for tuberculosis and its future demand in the pharmaceutical industry is expected. However, its chemical synthesis is not simple, because γ-D-glutamyl-L-tryptophan has several reactive groups and consists of D- and L-amino acids, connected by a γ-glutamyl linkage. In contrast, γ-D-glutamyl-L-tryptophan is an ideal compound to produce using bacterial GGT (Suzuki et al., 2004b). Using D-glutamine as a γ-glutamyl donor, γ-glutamylglutamine is not synthesized as a by-product. And L-tryptophan being a basic amino acid is a good γ-glutamyl acceptor.

Superiority of enzymatic production using bacterial GGT

Our enzymatic method of producing γ-glutamyl compounds using bacterial GGT is superior to other methods in various ways. First of all, since amino acids have several reactive groups, blocking and deblocking of the reactive groups are required for chemical production of γ-glutamyl compounds. However, GGT only connects the γ-carboxyl group of glutamic acid and amino group of the acceptor. Second, no energy source such as ATP is required because GGT is a transferase and not a synthetase. For example, production of theanine using bacterial glutamine synthetase and of various γ-glutamyl compounds using bacterial γ-glutamylcysteine synthetase was reported (Yamamoto et al., 2005; Nakayama et al., 1981). These enzymes require ATP as an energy source, but they are also inhibited by the by-product ADP. Therefore, sophisticated designs were invented to supply a small amount of ATP continuously by combining the reactions with the ATP-regenerating system. Since GGT does not require ATP, the GGT method is quite simple and just mixing GGT with substrates is enough. Third, among GGTs from various sources, bacterial GGTs are preferable. Mammalian GGTs prefer glutathione as a γ-glutamyl donor, while bacterial GGTs can utilize less expensive glutamine as well as glutathione (Suzuki et al., 1986a; Minami et al., 2003). Moreover, bacterial GGTs have a broad substrate specificity for γ-glutamyl acceptors and various γ-glutamyl compounds can be produced. Bacterial GGTs are either periplasmic (Suzuki et al., 1986b) or extra-cellular (Minami et al., 2003) existing as soluble and non-glycosylated protein, while eukaryotic GGTs are membrane-bound glycosylated proteins (Taniguchi and Ikeda, 1998). Therefore, bacterial GGTs are much easier to prepare and in fact, can be purified from overproducing strains by means of a simple two-step method (Suzuki et al., 1988; Claudio et al., 1991; Minami et al., 2003) and so a large amount of GGT is readily available. Lastly, various γ-D-glutamyl compounds can be produced efficiently and stereo-specifically using D-glutamine as a γ-glutamyl donor (Suzuki et al., 2003). It is difficult to synthesize γ-D-glutamyl compounds by other enzymes, such as glutamine synthetase, because most enzymes are L-amino acid-specific.

Two enzymatic methods to produce L-alanyl-L-glutamine using novel enzymes have been reported. With one method, alanine should be derivatized to *O*-methylester and the enzyme which catalyzes the formation of a peptide linkage between alanine-*O*-methylester and glutamine releases methanol (Yokozeki and Hara, 2005). The requirement of an extra step, derivatization of alanine, and the formation of methanol as a by-product are the disadvantages of this method. The other method uses L-amino acid α-ligase to produce L-alanyl-L-glutamine (Hahimoto et al., 2004). Since the enzyme is a ligase, it requires ATP and coupling with an ATP-regenerating system is required as discussed above. Another point is that this enzyme can also produce α-L-glutamyl-L-alanine as a by-product. Therefore, the production of γ-L-glutamyl-L-glutamine using bacterial GGT is much simpler. This is another advantage of γ-L-glutamyl-L-glutamine over L-alanyl-L-glutamine to stabilize L-glutamine.

Challenge to the more effective production of γ-glutamyl compounds

Improvement of the production of the bacterial GGT

The enzymatic method used to produce γ-glutamyl compounds with bacterial GGT is a simple one-step process which does not require any energy source. The most expensive step in this process is the preparation of GGT, even if the enzyme is immobilized or reused. Bacterial GGTs have been purified from overproducing strains by means of a simple two-step method. *E. coli* GGT has been purified from an *E. coli* strain harboring a pUC plasmid containing the *E. coli ggt* gene. GGT was overproduced using the gene dose effect of a high copy number plasmid, pUC, and purified by ammonium sulfate fractionation and chromatofocusing (Suzuki et al., 1988). It would be helpful if the production of bacterial GGT could improve further. When the *ggt* gene was subjected to the control of the T7 promoter, a large amount of GGT-precursor precipitated as an inclusion body. Therefore, the coding region of the large and small subunits of the *E. coli ggt* gene without the signal peptide region was inserted between the *Sph*I and *Pst*I sites of pQE-80 L (Qiagen). In this construct, the *ggt* gene is located downstream of the T5 promoter and is under the control of *lacO* regulation, and MRGSH$_6$GSAC was attached to the N-terminal of the large subunit. After induction with 0.5 mM IPTG, the growth temperature was shifted from 37 to 20 °C and kept at 20 °C for 8 h. GGT was purified from the cell-free extracts using a Ni affinity column. Employing this new purification method, the yield of purified GGT obtained per liter of culture broth increased 8-fold (Suzuki and Yamada, unpublished data).

Activation of transpeptidation reaction by cations

The amount of GGT added to the reaction mixture could be reduced if the GGT activity could be increased. It was found that the transpeptidation reaction of *E. coli* GGT is activated by several cations at alkaline pH (Suzuki and Kato, unpublished data). Effects of the addition of monovalent and divalent cations were measured at 0.5 M using the standard assay method (Suzuki et al., 1986a). This was the optimum NaCl concentration for activating the transpeptidation. All cations tested activated the reaction (Table 2). The optimum concentrations of $MgCl_2$ and $CaCl_2$ were 50 and 100 mM, respectively. The addition of such a small amount of these divalent cations activated the transpeptidation reaction more than did adding 0.25–0.5 M monovalent cations. Divalent cations may change the conformation of the GGT molecule, making it more favorable for the transpeptidation reaction. It should be pointed out that GGT belongs to the N-terminal nucleophile hydrolyases (Brannigan et al., 1995; Inoue et al., 2000; Okada et al., 2006) and not to the metallopeptidases.

Whether the addition of cation is also effective in an enzymatic method to produce γ-glutamyl compound using bacterial GGT was tested. $MgCl_2$ was chosen because γ-glutamyl compounds are often intended for use as food additives and medications, and cations, which are not appropriate, should thus not be used. $MgCl_2$ has traditionally

Table 2. Effect of monovalent and divalent cations on the transpeptidation reaction of *E. coli* GGT

Cation[a]	Relative activity (%)[b]	Cation[a]	Relative activity (%)[b]
None	100	$MgCl_2$	108
LiCl	194	$MgCl_2$ (50 mM)	194
NaCl	190	$CaCl_2$	105
KCl	175	$CaCl_2$ (100 mM)	224
RbCl	175	$MnCl_2$ (5 mM)	134
CsCl	174	$CoCl_2$ (1 mM)	112

[a] The cations were added to the reaction mixture to 0.5 M unless otherwise stated

[b] The transpeptidation activity without any cation (0.21 U/ml) is expressed as 100%. The transpeptidation activity was measured by a photometric method using γ-G*p*NA and Gly-Gly as substrates as described previously (Suzuki et al., 1986a)

[c] $MnCl_2$ and $CoCl_2$ were added at lower concentrations because they formed precipitates at higher concentrations

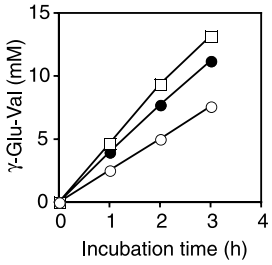


Fig. 4. Effect of the addition of $MgCl_2$ on the production of γ-glutamylvaline. The reaction was carried out with 20 mM L-glutamine and 300 mM L-valine at pH 10 and 37 °C. The concentration of GGT was 0.02 U/ml (○) and 0.04 U/ml (□). Fifty mM of $MgCl_2$ was added to one of the reaction mixtures containing 0.02 U/ml GGT (●). After 0, 1, 2, and 3 h incubation, 100 µl of sample was subtracted and the reaction was terminated. The concentration of γ-glutamylvaline was measured by reverse phase HPLC (Suzuki et al., 2004a)

been used as a coagulant for tofu (soybean curd), and is both cheap and well accepted as a food additive in Japan. NaCl and KCl are inexpensive and edible, but to obtain comparable activation to $MgCl_2$, extremely large amounts should be added, making it difficult to purify the intended γ-glutamyl compounds from the reaction mixture. Therefore, the effect of the addition of 50 mM $MgCl_2$ to the reaction mixture for production of γ-glutamylvaline was evaluated (Fig. 4). In the optimum reaction mixture, the concentration of GGT was 0.04 U/ml (Suzuki et al., 2004a). When half this amount was used, the production of γ-glutamylvaline was reduced to about 55% of that obtained with the optimum reaction conditions. When 50 mM $MgCl_2$ was added to the reaction mixture with 0.02 U/ml GGT, the production recovered to about 85% of that under the optimum conditions. The results clearly indicate that the addition of $MgCl_2$ can compensate for the low enzymatic activity of GGT.

Conclusions

As described in this review, several γ-glutamyl compounds, which have been studied, have attractive features. It is speculated that other γ-glutamyl compounds also have characteristics that may benefit humans. Nonetheless, only a small number of γ-glutamyl amino acids have been studied. This is mainly because these γ-glutamyl compounds are not available from commercial sources. Applying the enzymatic method for the production of γ-glutamyl compounds using bacterial GGT, we are now able to synthesize various attractive γ-glutamyl compounds readily. γ-Glutamyl compounds are available and promising materials with great potential.

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