Gamma-glutamyl dipeptides and amines

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γ-Glutamyl dipeptides (γ-Glu AA) are defined in this context as the compounds derived by the acylation of an amino acid (AA) through the γ-carboxyl carbon of L-glutamic acid. The resulting amide linkage has sometimes been referred as a pseudo-peptide bond. The general structure of a typical γ-glutamyl dipeptide is given in Figure 1. The aim of this short review is to highlight the modification of properties of the amino acid which is γ-glutamylated. Also a brief review of the chemistry leading to γ-glutamyl dipeptide in a practical and efficacious way requiring the least number of protection / deprotection steps. The practical applications of the resultant products are discussed.

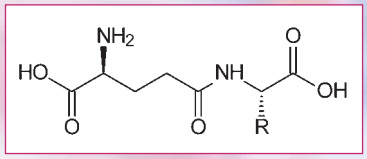

Figure 1 – γ-Glutamyl amino acid (γ-Glu AA or γ-glutamyl dipeptide)

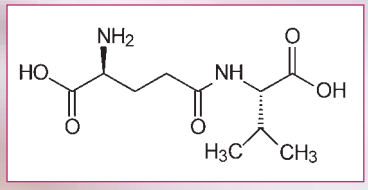

Figure 2 – γ-Glutamylvaline (γ-Glu Val)

Enzymes belonging to the class called γ-glutamyl transpeptidases lead either to the formation of this pseudopeptide bond or hydrolysis of the γ-glutamyl dipeptide into its constituent amino acids depending on conditions.

γ-GLUTAMYL SUBSTRUCTURE CHANGING THE BITTERNESS OF AMINO ACIDS

L-amino acids with branched chain, basic groups or aromatic substructure are known to be bitter. Hence when amino acid supplements need to be taken orally, the bitterness of the amino acids is a property that needs to be minimized or masked. While addition of sweeteners or flavorings alleviate this problem, it has been found that γ-glutamylation of amino acids abolished or reduced their bitterness to a significant extent. Suzuki *et al* found (1) that the bitter taste of valine was highly attenuated on transformation to its γ-glutamyl derivative (Figure 2). Similar glutamylation has been carried out on isoleucine and tyrosine.

N^{ε}-(γ-GLUTAMYL)LYSINE AS SOURCE OF LYSINE, IN BREAD AND NOODLES

N$^{\varepsilon}$-(γ-glutamyl)lysine (Figure 3) is an important amino acid component forming part of food products. Transglutaminase (Tgase, EC 2.3.2.13) is a transferase widely distributed in microbes, mammals, plant and fish that catalyzes the acyl transfer reaction between γ-carboxyl group of a glutamic acid residue with ε-amino group of a lysine moiety in an intermolecular or intramolecular fashion. This results in structural rigidity of the protein in edible food. Nutritional improvement of bread has been noticed with N^{ε}-(γ-glutamyl)lysine (2) wherein N^{ε}-(γ-glutamyl)lysine serves as a nutritional source of lysine. In another study it was found that scanning electron microscopy indicated that the physical properties of dry noodles were improved

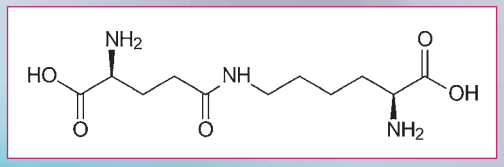

Figure 3 – N^{ε}-(γ-glutamyl)lysine

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through the formation of cross-links, Nε-(γ-glutamyl)-lysine, brought about by MTGase (3).

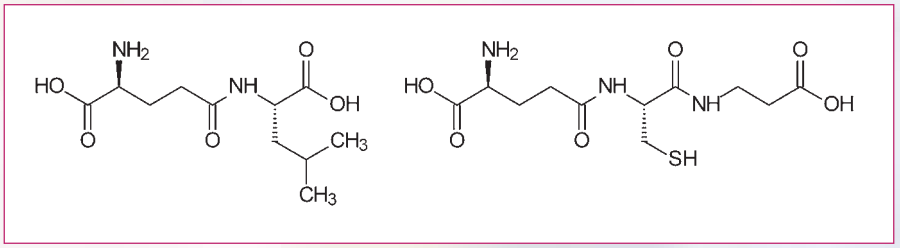

Figure 4 – γ-Glutamylleucine and homoglutathione

γ-GLUTAMYL AMINO ACIDS AS KOKUMI COMPOUNDS

Peas, lentils and other podded plants are served with meat in several cuisines around the world. Addition of a nearly tasteless aqueous extract from beans (*Phaseolus vulgaris)* to chicken broth enhanced its mouthfulness and complexity. It induced a much more long-lasting savory taste sensation on the tongue. Chromatographic isolation and detailed NMR studies on this extract from *Phaseolus vulgaris* led to the identification of γ-glutamylleucine, γ-glutamylvaline and γ-glutamylcysteinyl-β-alanine (homoglutathione) (Figure 2 and Figure 4) as the molecules responsible for this taste modifying effect. Very interestingly, detection threshold values of these γ-glutamyl peptides decreased manifold in the presence of savory matrix such as sodium chloride or chicken broth in enhancing long-lastingness of the savory taste, mouthfulness and complexity.

Molecules inducing this type of mouthfulness and thickness and increasing continuity of food taste perception have been termed by the Japanese as "kokumi" flavor compounds and such properties are ostensibly conferred by the γ-glutamyl residue on the amino acids (4). Glutathione, itself a γ-glutamyl peptide, γ-Glu-Cys-Gly, is also a "kokumi" compound. Another mouthfulness enhancing "kokumi" compound with a γ-glutamyl substructure is γ-glutamyl-*trans*-*S*-propenyl-L-cysteine sulfoxide (Figure 5) occurring in garlic and onion.

Occurrence of these γ-glutamyl "kokumi" compounds attest to the use of beans, garlic, onion being served often with meat dishes for enhancing the sensory perception as well as a longer lasting food sensation.

γ-GLUTAMYL SUBSTRUCTURE CHANGING THE OLFACTORY PERCEPTION

Selenium amino acids rank among the top of the list on selenium supplements. Selenium has been recognized as an essential micronutrient the lack of which leads to various disease states (5), ascribed to the deficiency of selenium containing amino acids in the body. Certain selenium compounds are

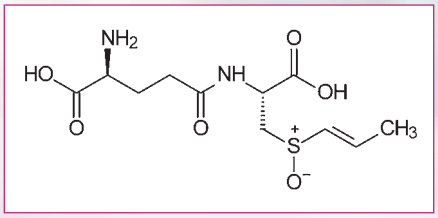

Figure 5 – γ-Glutamyl-trans-S-propenyl-L-cysteine sulfoxide

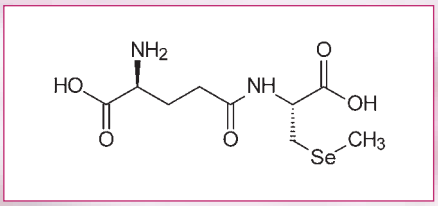

Figure 6 – γ-Glutamyl-Se-methyl-L-selenocysteine

offensive in smell and may become a deterrent factor for oral and topical use. For example, Se-methyl-L-selenocysteine has an odor associated with it but the γ-glutamyl derivative is practically odorless (Figure 6). Still the biological characteristics of Se-methyl-L-selenocysteine are not considerably changed by this structural variation. It has been well recognized that γ-glutamyl-Se-methyl-L-selenocysteine is a carrier molecule as effective as its parent, Se-methyl-L-selenocysteine, in its anticancerous properties (6).

This improvement of olfactory property has another important application in cosmetics. While the not-so-acceptable smell of some of the selenium compounds may have contributed to a slightly muted acceptance of selenium compounds in cosmetic industry, olfactory improvement by γ-glutamylation should lead to a favorable acceptance of these compounds by the cosmetic industry.

γ-GLUTAMYLATION AND PHYSICAL PROPERTIES

Since γ-glutamylation introduces more polar functional groups with hydrophilic characteristics, it can be anticipated that the solubility of γ-glutamyl amino acid in water may be enhanced compared to the corresponding underivatized amino acid. Such has been the case in the duo, Se-methyl-L-selenocysteine and its glutamylated derivative, γ-Glutamyl-Se-methyl-L-selenocysteine. The solubility of the L-Se-methylselenocysteine was ca. 10% with dissolution occurring in two hours in water. The solubility of γ-L-glutamyl-L-Se-methylselenocysteine was ca. 25% with dissolution occurring in thirty minutes in water. This solubility modifying characteristic has important ramifications in the formulation of these materials.

MISCELLANEOUS EXAMPLES

Amino acids such as γ-glutamyl-taurine (Glutaurine) isolated from bovine parathyroids has been reported to influence the metabolism of Vitamin A and to possess radioprotective properties in addition to other useful therapeutic properties. Again such an array of beneficial properties would appear to result from the useful modification of the taurine structure with γ-glutamyl group (7).

γ-D-Glutamyl-L-tryptophan (SCV-07) is a prospective medicine for the treatment of tuberculosis, that reached up to phase two clinical trial. Here the optical antipode is used (8). Another stellar example is Theanine. Even though it is not a γ-glutamyl aminoacid, rather a γ-glutamyl ethyl amine, the γ-glutamyl structural fraction is the most important feature of the molecule.

γ-Glutamyl derivatives have been well recognized for their immunomodulating properties. Both amino acid and amine analogs have been described in the literature.

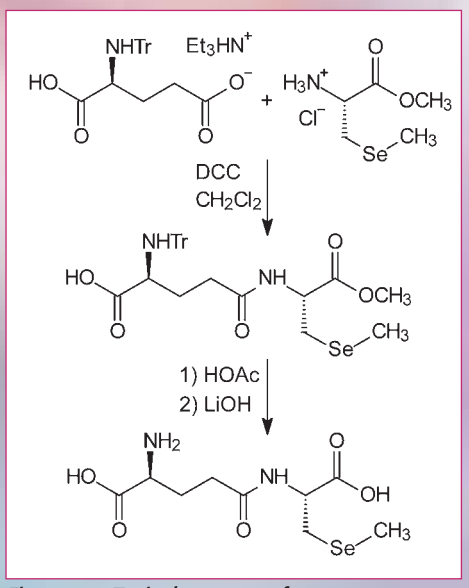

Figure 7 – Typical sequence for γ-glutamylation of amino acids

CHEMISTRY OF γ-GLUTAMYLATION

γ-Glutamyl group can be incorporated onto a typical amino acids in a several ways. Basically this usually requires the protection of two of the three carboxylic groups in the reactant molecules as esters – leaving of course the third γ-COOH of the glutamic acid free.

Also the amino group of the L-glutamic acid residue should be suitably protected. Figure 7 shows a sequence wherein only two protecting groups are used. The trityl group on the glutamic acid function plays a dual role wherein it protects the amino group as well as rendering the neighboring -COOH sterically unreactive. The carboxyl of the other component, namely, Se-methyl-L-selenocysteine is protected as a methyl ester. After the formation of pseudo-peptide bond, the two protecting groups need to be removed sequentially without affecting the amide linkage (9).

However the availability of N-phthaloyl-L-glutamic anhydride (NPGLA) (Figure 8) in commercial quantities in high chemical and chiral purity has changed this scenario (10). The γ-glutamylation sequence becomes simpler and higher yielding when N-phthaloyl-L-glutamic anhydride is used as the γ-glutamylating agent.

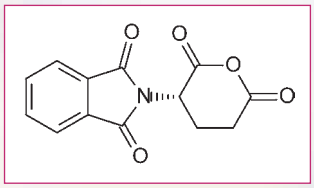

Figure 8 – N-phthaloyl-L-glutamic anhydride (NPGLA)

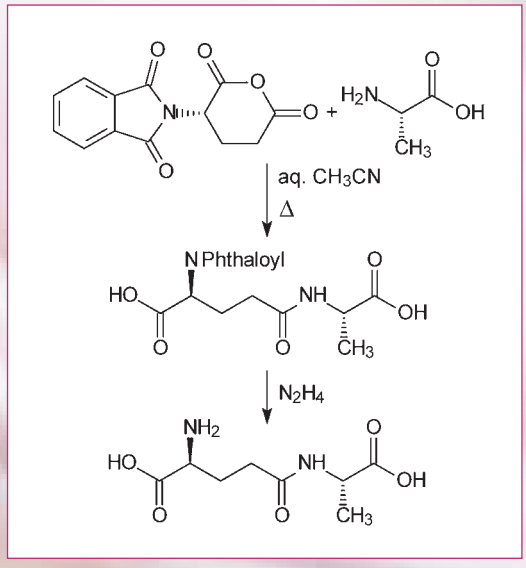

Figure 9 – Reaction sequence for γ-glutamylation using NPGLA

The amino acid, that needs to be γ-glutamylated, can be used directly without any protecting groups. A solvent system incorporating an aqueous polar solvent can be employed. The products are free from any possible racemization. Amines also can be employed on their conversion to γ-glutamyl derivatives. Phthaloyl group can be removed by standard methods such as use of hydrazine (Figure 9).

In the synthesis of γ-glutamyl-marasmine, NPGLA has been used effectively (11). An enantioselective synthesis of chloroquine and the establishment of configuration used NPGLA as a pivotal chiral agent (12). Enzymatic methods of introduction of γ-glutamyl group have also been used (1). A bacterial γ-glutamyltranspepdidase enzyme was used. This enzymic technology still has to reach industrial levels since only low concentrations of substrates only could be used.

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