Note

Formation of γ -Glutamyl Peptides by Glutaminase of *Aspergillus oryzae*

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Fig. 1. Detection of γ -Glutamyl Peptides by High Voltage Paper Electrophoresis.

(a) Detection of γ -glutamylglutamine and γ -glutamylglutamic acid. Lane 1, authentic γ -glutamylglutamine, γ -glutamylglutamic acid, glutamine and glutamic acid; lane 2, reaction mixture at 0 hr incubation; lane 3, reaction mixture at 8 hr incubation; lane 4, reaction mixture at 8 hr incubation, with hydrolysis with the commercial GGT.

(b) Detection of γ -glutamylvaline. Lane 1, authentic γ -glutamylglutamine, γ -glutamylglutamic acid, glutamine, glutamic acid and valine; lane 2, reaction mixture at 0 hr incubation; lane 3, reaction mixture at 8 hr incubation; lane 4, reaction mixture at 8 hr incubation, with hydrolysis with the commercial GGT.

Twenty μ l of each sample was applied to Whatman 3MMChr paper and then electrophoresed at a potential gradient of 72 V/cm for 45 min. The composition of the reaction mixture and the reaction conditions are given in the text. The hydrolysis conditions were given previously.⁶⁾

In the course of a study on glutaminase of koji mold, we found that the enzyme purified from koji mold, *Aspergillus oryzae* MA-27-IM, could catalyze the γ -glutamyltranspeptidation (GGT) reaction.¹⁾ This was the first reported case of a filamentous fungus, though GGT reactions in animals and bacteria have been reported.^{2~5)} In this reaction, the γ -glutamyl moiety of L- γ -glutamyl-*p*-nitroanilide was transferred to a peptide, glycylglycine, to yield γ -glutamylglycylglycine. Furthermore, using L-glutamine as a γ -glutamyl donor and glycylglycine as the acceptor, we confirmed the formation of γ -glutamylglycylglycine.⁶⁾

On the other hand, in the process of food production involving koji molds, such as in soy sauce and miso fermentations, it is known that many kinds of peptides and amino acids, including glutamine, are liberated in large amounts from material proteins into the mash through the actions of proteinases and peptidases.⁷⁾ This observation suggests us that the GGT reaction might occur between glutamine and peptides or amino acids, and that γ -glutamyl peptides might be formed in the mash.

Thus, taking the above knowledge and the results of our previous study into consideration, in this study, we investigated the formation of γ -glutamyl peptides from L-glutamine and amino acids by using the crude and the purified glutaminase from *A. oryzae* MA-27-IM. Preparation of the crude and purified enzymes, and assaying of GGT activity were performed as described in our previous papers.^{1,6)}

First, the enzyme reaction was carried out using L-glutamine only as the substrate. The reaction mixture contained 50 mM L-glutamine, 100 mM Tris-HCl buffer (pH 8.5) and 0.36 units/ml of crude enzyme, and the reaction was carried out at 30°C for 8 hr. The crude enzyme preparation was the 85% ammonium sulfate saturation fraction of a water extract of wheat bran koji obtained with *A. oryzae* MA-27-IM. The reaction mixture was analyzed by high voltage paper electrophoresis (HVPE), which was performed on Whatman No.

3MMChr paper (18 $\times$ 55 cm) dipped in a solvent system consisting of pyridine, glacial acetic acid and water (1:10:289, v/v, pH 3.6),⁸⁾ with a gradient potential of 72 volts/cm for 45 min. As shown in Fig. 1(a), lane 3, the reaction mixture exhibited two unknown ninhydrin positive spots, compounds A and B, besides spots of glutamine and glutamic acid. Compounds A and B showed the same migration as a commercial γ -glutamylglutamine and a commercial γ -glutamylglutamic acid, respectively (Fig. 1(a), lanes 1 and 3). On hydrolysis with a commercial GGT (from bovine kidney, Sigma Co., Ltd.), the ninhydrin staining of compounds A and B became faint (Fig. 1(a), lane 4), suggesting that both compounds were γ -glutamyl peptides. The hydrolysis conditions were given previously.⁶⁾

The two compounds were isolated and identified as follows. One ml of the reaction mixture was subjected to HVPE (100 μ l per paper). After electrophoresis, both sides

of the paper were cut out and then the positions of compound A and B were determined by the ninhydrin method. The paper strips corresponding to compounds A and B were cut out of the paper and extracted with distilled water. The extracts were concentrated in a rotary evaporator to a small volume. Each extract gave a single peak on a chromatogram with an amino acid analyzer (Kyowa Seimitsu K-101AS). For identification, the two compounds were hydrolyzed with the commercial GGT and HCl as described previously.⁶⁾ The hydrolysis products were analyzed with the amino acid analyzer. Compound A gave glutamic acid and glutamine in the molar ratio of 1.0 to 1.0 on hydrolysis with the commercial GGT and only glutamic acid on HCl hydrolysis. Compound B yielded only glutamic acid with both hydrolyses. Thus, we identified compound A as γ -glutamylglutamine and compound B as γ -glutamylglutamic acid. γ -Glutamylglutamine seemed to be formed from L-glutamine itself through the transpeptidation reaction. On the other hand, γ -glutamylglutamic acid seemed to be formed through the same reaction between L-glutamine and glutamic acid produced from L-glutamine through the hydrolysis reaction.

Secondly, the transpeptidation between L-glutamine and L-valine was carried out and the products were identified as follows. The reaction was performed with the addition of 50 mM L-valine as an acceptor in the reaction system mentioned above. For the reaction mixture, a new ninhydrin positive compound (compound V, indicated by an arrow in Fig. 1(b)), *i.e.*, besides γ -glutamylglutamine and γ -glutamylglutamic acid, was detected on the anode side of the HVPE paper (Fig. 1(b), lane 3). After treatment of the reaction mixture with the commercial GGT, the ninhydrin staining of compound V as well as those of γ -glutamylglutamine and γ -glutamylglutamic acid became faint (Fig. 1(b), lane 4). Compound V was purified and identified in the same way as mentioned above. Compound V yielded valine and glutamic acid in the molar ratio of 1.0 to 0.95 on hydrolysis with the commercial GGT and in the molar ratio of 1.0 to 1.05 with HCl. Thus, compound V was identified as γ -glutamylvaline.

The formation of γ -glutamyl peptides from L-glutamine and various amino acids is summarized in Table I. L-Serine, L-valine L-glutamic acid and L-glutamine itself proved to be better acceptors. Similar results were obtained (data not shown) using the same reaction system in which the enzyme was substituted by the purified glutaminase. In this study, it was confirmed that the γ -glutamyl-transpeptidation reaction can take place between L-glutamine and an amino acid through the action of a glutaminase from a koji mold. This finding suggests the

Table I. γ -GLUTAMYL PEPTIDE FORMATION BY *Aspergillus oryzae* GLUTAMINASE^a

Acceptor (50 mM)	γ -Glutamyl peptide formed ^b	Mobility ^c	Amount (mm) ^d
L-Gln ^e	γ -GluGln	6.3	4.3
L-Glu	γ -GluGlu	6.9	4.6
L-Ala	γ -GluAla	4.9	1.7
L-Phe	γ -GluPhe	5.0	2.5
L-Ser	γ -GluSer	8.8	3.8
L-Ile	γ -GluIle	4.0	2.3
L-Val	γ -GluVal	4.3	3.5
Gly	γ -GluGly	5.6	2.2

^a Crude enzyme was used.

^b γ -GluGln and γ -GluGlu were regularly formed from L-glutamine.

^c All γ -glutamyl peptides listed here migrated to the anode side of the paper. The mobility of L-glutamic acid was taken as 1.0.

^d The amount of a γ -glutamyl peptide was determined with an amino acid analyzer on the assumption that it has the same extinction coefficient as glutamic acid.

^e Only L-glutamine (50 mM) was used as the substrate.

occurrence of the transpeptidation reaction and the formation of γ -glutamyl peptides in the mash in fermentative food production processes such as soy sauce and miso production.

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