Short Paper

Determination of ϵ -(γ -Glutamyl)lysine in Several Fish Eggs and Muscle Proteins

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ϵ -(γ -Glutamyl)lysine (Glu-Lys) is an isomeric peptide bond formed between γ -carboxyamide group of glutamines and ϵ -amino group of lysines in proteins and/or peptides by transglutaminase (EC 2.3.2.13) reaction, and illustrated for simple polymerization as:

The presence of Glu-Lys has been reported widely in natural proteins and biological materials, such as fibrin,¹⁾ keratin,²⁾ skeletal myofibrils³⁾ and fish egg membranes.⁴⁾

Nio *et al.*⁵⁾ reported that protein solutions could be changed to gel by the Glu-Lys bonds. We, therefore, assume that Glu-Lys bond contents may be related to some rheological properties of protein gels. Recently, participation of transglutaminase reaction during the setting process in kamaboko (salted fish meat product, fish cake) manufacturing and correlation between Glu-Lys contents and gel strength has been elucidated.^{6,7)} We measured Glu-Lys contents in seven kamabokos purchased from local markets, and obtained the results that Glu-Lys was detected in all the samples (mean $\pm$ SD, 0.55 ± 0.35 $\mu\text{mol/g}$ protein).⁸⁾

In this short paper, we report Glu-Lys content in several fish eggs and muscle proteins. All samples were purchased from local supermarkets and listed in Table 1. Quantitation of Glu-Lys was performed according to the method of Griffin *et al.*⁹⁾ with slight modification.¹⁰⁾ Briefly, fish egg, except for “karasumi” and “caviar”, or ordinary dorsal muscle was freshly removed from fish and homogenized with five volumes of distilled water, then lyophilized. For salted and dried egg products, such as “karasumi” and “caviar”, they were homogenized as intact. Each lyophilized sample (15–20 mg of protein) was digested thoroughly and successively by pronase E, prolidase, leucine aminopeptidase and carboxypeptidase A to obtain the

mixture of amino acids and Glu-Lys in 0.1 M borate buffer (pH 8.0). The digested samples were preliminarily fractionated to remove interfering compounds by reversed phase high-performance liquid chromatography (RP-HPLC) with Inertsil ODS-2 column (G-L Science Co., Ltd., Tokyo, Japan). The fraction containing Glu-Lys was collected and derivatized with *o*-phthalaldehyde (OPA). The OPA-labeled Glu-Lys was injected into RP-HPLC with Zorbax BP-C8 column (G-L Science) and detected using a fluorescence detector (Ex 334 nm, Em 440 nm). The amount of Glu-Lys was determined with synthetic Glu-Lys as standard.

The results are summarized in Table 1. Glu-Lys was detected in all fish egg samples and the contents ranged from 0.12 to 2.04 $\mu\text{mol/g}$ protein, and on an average, 0.99

Table 1. ϵ -(γ -Glu)Lys Contents in Eggs and Muscles of fish and other marine animals

		($\mu\text{mol/g}$ of protein)	
Sample		ϵ -(γ -Glutamyl)Lysine	
		Eggs	Muscles
<i>Fish</i>			
Alaska pollack	<i>Theragra chalcogramma</i>	0.58	ND ^{a)}
Flatfish	<i>Limanda herzensteini</i>	0.49	— ^{b)}
Flying fish	<i>Prognichthys agoo</i>	1.47	—
Herring	<i>Clupea pallasii</i>	0.91	0.23×10^{-2}
Horse mackerel	<i>Trachurus japonicus</i>	—	0.73×10^{-2}
Longfin smelt	<i>Spirinchus lanceolatus</i>	1.21	—
Red Salmon	<i>Oncorhynchus keta</i>	0.12	0.15×10^{-2}
Sandfish	<i>Arctoscopus japonicus</i>	1.12	—
Sardine	<i>Sardinops melanosticta</i>	0.26	ND
Seabass	<i>Lateolabrax japonicus</i>	—	ND
<i>Egg product</i>			
Caviar	<i>Acipenser mikadoi</i>	1.74	—
Karasumi	<i>Mullet mugilcephalus</i>	2.04	—
<i>Mollusk</i>			
Octopus	<i>Octopus vulgaris</i>	—	0.14×10^{-2}
Squid	<i>Doryteuthis bleekeri</i>	—	0.19×10^{-2}
<i>Crustacean</i>			
Crab	<i>Erimacrus isenbeckii</i>	—	0.34×10^{-2}
Shrimp	<i>Metapenaeus joyneri</i>	—	0.38×10^{-2}
Average		0.99	0.22×10^{-2}

^{a)} ND: Not detected.

^{b)} —: Not determined.

$\mu\text{mol/g}$ protein. The sample having the largest amount of Glu-Lys was "karasumi" ($2.04 \mu\text{mol/g}$ protein) and the second was "caviar" ($1.74 \mu\text{mol/g}$ protein). These samples were both fish egg products. In fresh eggs, flying fish had the largest amount ($1.47 \mu\text{mol/g}$ protein) and red salmon had the smallest value ($0.12 \mu\text{mol/g}$ protein). There were little relation between these values and environmental conditions where fish live. In salmon, the contents of Glu-Lys were relatively small in both egg and muscle compared to other fish. Recently, a transglutaminase inhibitor was found in salmon muscle water-soluble fraction,* thus some relation may be present the reason for the low Glu-Lys content in salmon.

In contrast, Glu-Lys was not detected in dorsal muscles of some fish species, such as Alaska pollack, sardine and sea bass. In other fish species, such as herring, salmon and horse mackerel, detectable amounts of Glu-Lys were observed, but their amounts were less than one hundredth of those of fish eggs. As for mollusks and crustaceans muscle protein, all the samples had detectable amounts of Glu-Lys, but the contents of Glu-Lys were small as well as fish muscle protein.

Our results, therefore, indicate that Glu-Lys contents in the fish eggs are much larger than those in muscle proteins regardless of fish species. We also detected the transglutaminase activity in water-soluble fractions of fish egg homogenates (data not shown) and this suggests a relationship between the egg development and Glu-Lys. Thus, the reason that fish eggs having a larger Glu-Lys content than that of muscle protein may be the participation of Glu-Lys in formation of egg cell membrane and stabilization of the membrane protein by the Glu-Lys isopeptide bond.

That the Glu-Lys content in "karasumi" and "caviar"

were about twice as large as than that of some other raw fish eggs is particularly interesting in relation to the transglutaminase reaction during manufacturing process of fish egg products.

Finally, we are undertaking an investigation whether a correlation present between rheological properties of fish egg products, Glu-Lys contents, and transglutaminase activity. That is, we presume intrinsic transglutaminases in fish ovarium or eggs react and resulting Glu-Lys bond increases during manufacturing process of fish egg products and exerts some influence on their rheological properties.

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