A Novel Bitter Amino Acid, Pulcherrimine Isolated From Sea Urchin Ovaries: Relationship Between Maturation And Their Contents

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Abstract

The present study was undertaken to unders the properties and origins of the bitter substance in the sea urchin gonads and the relationship between maturation and their contents. The frequency of occurrence of bitter-tasting sea urchins collected in the sea off Iwaki, Fukushima Prefecture was examined first. The bitterness was found to be specific to mature ovaries. From the mature ovaries, a bitter substance was isolated and the structure was determined to be 4*S*-(2'-carboxy-2'*S*-hydroxy-ethylthio)-2*R*-piperidinecarboxylic acid, being a novel sulfur-containing amino acid, named pulcherrimine (Pul). Subsequently, we investigated the seasonal changes in the frequency of occurrence of mature female sea urchins and the frequency distribution of Pul contents among the mature female individuals at Iwaki, Fukushima Prefecture. Many mature individuals with high Pul-containing ovaries were found in all months examined in this area. From these findings, it was concluded that the presence of mature individuals with a high amount of Pul in all seasons is the major reason why sea urchins are hardly utilized as food in this area. In Mikuni, Fukui Prefecture, we investigated the relationship between the reproductive cycle and content of Pul in the ovaries of the sea urchin. From May to September, most of the individuals had immature gonads (stage 1), in which Pul was not detected. Many individuals were in stage 2 (growing) in October, and in stage 3 (pre-mature) in November. Pul content in the ovaries gradually increased during this period. In December and February, all the individuals were in stage 4 (mature). After the rapid decrease in GI, many individuals were in stage 5 (spent) in

March. The Pul content was high in the mature and spent ovaries. These results indicate that Pul accumulates in ovaries with the progress of oogenesis.

Introduction

The sea urchin, *Hemicentrotus pulcherrimus* is widely distributed in Japanese costal areas. It is one of the most important fishery products on the southwestern coasts of Japan, including Fukui Prefecture. However, sea urchins whose gonads taste extremely bitter are often found in the catch in the Tohoku area, including Fukushima Prefecture. Such sea urchins are not acceptable as food and have no commercial value. The present study was undertaken to examine the bitter substance in the sea urchin gonads and the relationship between maturation and their contents.

1. Isolation and structure elucidation of Pul from the sea urchin ovaries distributed in Iwaki

1.1 Frequency of occurrence of the bitter-tasting sea urchin in Iwaki

Sea urchins were collected from the sea off Iwaki, Fukushima Prefecture in March 1996 and March 1997. The numbers of specimens collected were 94 and 99, respectively. All specimens were judged to be mature, and their sex was readily distinguished by the oozed gametes (Fuji 1960). Ninety-five percent of the female individuals had bitter ovaries, while none of the male individuals had bitter testes. In March 1997, all of the urchins of which the gonads gave a bitter taste were found to be female. These results suggest that the bitterness of the sea urchin is specific to the mature ovaries (Murata *et. al.* 1998).

1.2 Isolation and structure elucidation of Pul from the sea urchin ovaries

The bitter substance was isolated from mature sea urchin ovaries using ODS column chromatography, Sephadex G-10 gel filtration, and Re-HPLC. The structure of the bitter substance was determined to be *S*-(2'-carboxy-2'*S*-hydroxyethylthio)- 2*R*-piperidinecarboxylic acid, a novel sulfur-containing amino acid by HRFABMS data, and ^{1}D and ^{2}D NMR spectral data (Murata and Sata 2000). It was named pulcherrimine (Pul) after the scientific name for *H. pulcherrimus* (Fig. 1).

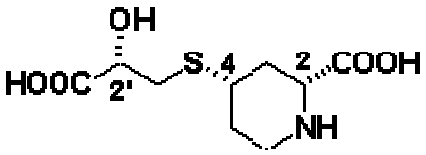

Figure 1. Pulcherrimine

The stereochemistry of Pul was determined by NOE experiments, chiral HPLC analysis and modified Mosher method (Murata and Sata 2000; Sata *et.al*. 2002).

1.3 Seasonal changes in the frequency of occurrence of mature female sea urchins and Pul content in the ovary of the sea urchin

At intervals of every three months from November 1998 to November 1999, 100 sea urchins were randomly collected from the sea off Iwaki. The sampled sea urchins were divided into mature and immature individuals. Mature individuals were defined in this study as those with gametes, which ooze from the gonads. Immature individuals were defined as those with gametes, which do not ooze from the gonads. The sex of the mature individuals was identified from the oozed gametes (Fuji 1960). Table 1 shows the frequency of mature male, female and immature sea urchins (Murata *et. al.* 2002). In November 1998 and February 1999, all of the sea urchins examined were mature, and thus the sex of each individual was easily distinguished. In May and August 1999, the frequency of immature individuals was relatively high: 20% and 60%, respectively. This suggests that mature individuals occur in all seasons in the sea off Iwaki.

Table 1. The frequency of occurrence of mature male and female, and immature sea urchin

	Mature		Immature
	Male	Female	
November 1998	56	44	0
February 1999	57	43	0
May 1999	50	30	20
August 1999	20	21	59
November 1999	56	40	4

Seasonal changes in the GI of each sex of the sea urchin are shown in Fig. 2 (Murata *et. al.* 2002). The GI values showed a large variation among the mature

specimens in each season. The fact indicates that the maturation process of the sea urchins might vary among individuals. The mean GI values of gonads were observed to decrease significantly during the period between February and May 1999, being at the lowest values in May 1999, and to increase thereafter. This change in the GI suggests that the major spawning season is in the period from February to May.

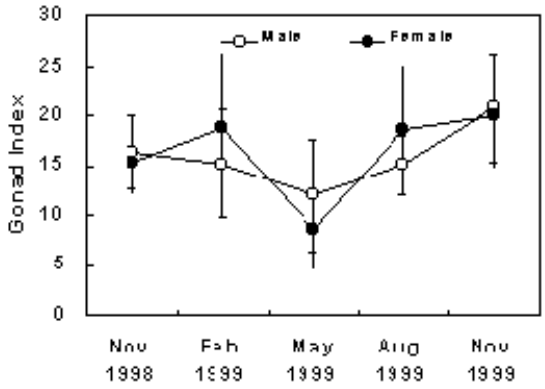

Figure 2. Changes in GI of mature male and female individuals

From the variation of the individual GI values and the presence of mature individuals in all seasons, the reproductive cycle of this species of sea urchin is unclear, and the occurrence of the mature stage extends over a long term, at least, in this sea area.

Twenty specimens were randomly selected from each of mature ovaries and testes and analyzed for Pul content (Murata *et. al.* 2001) in each month. No Pul was detected in mature testes, and they had no bitter taste. Changes in the mean Pul content in ovaries are shown in Fig.3. Many mature females with high Pul-containing ovaries were found in all seasons. Pul levels of ovaries were the highest in February. However, mean Pul content of ovaries were more than 0.8 mg/100g in all seasons. From these findings, it was concluded that the presence Pul in mature female ovaries is the major reason that sea urchins are hardly utilized for food in the Iwaki area.

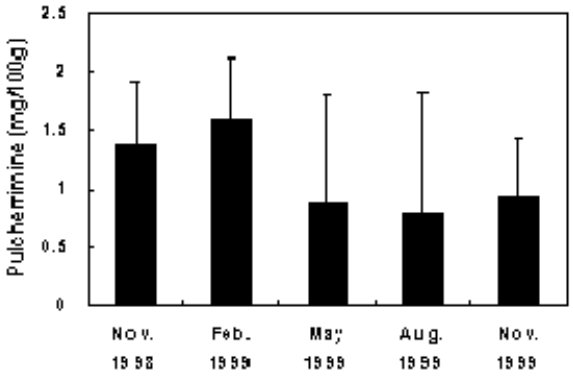

Figure 3. Seasonal changes in the mean Pul content in ovaries

2. Relationship between the reproductive cycle and the content of Pul in *H. pulcherrimus* ovaries

At intervals of one to three months from December 2000 to December 2001, 10-30 *H. pulcherrimus* were collected off Mikuni, Fukui Prefecture. The gonadal maturity of each individual was classified into the following stages initially proposed by Fuji (1960) and then modified by Unuma (2002).

Stage 1 (Recovering): Immature gonads before gametogenesis. Gonadal acini are filled with nutritive phagocytes (NPs). In females, a few young oocytes are present at the periphery of the acini. In males, haematoxylin-stained speckles, residue from phagocytized spermatozoa, are often present in NPs, whereas germ cells are difficult to be seen.

Stage 2 (Growing): Beginning of gametogenesis. Many developing oocytes or clusters of spermatogonia are present at the periphery of the gonadal acini.

Stage 3 (Pre-mature): Middle of gametogenesis. In the center of the gonadal lumina, NPs are replaced with ripe ova or spermatozoa.

Stage 4 (Mature): Fully-mature gonads at the end of gametogenesis. The gonadal lumina are filled with ripe ova or spermatozoa.

Stage 5 (Spent): After spawning, the gonadal lumina have numerous empty spaces

and a few residual ova or residual spermatozoa.

Changes in the GI of each sex are shown in Fig. 4. In 2001, the mean GI values of male and female individuals rapidly decreased from February to March, and then tended to increase.

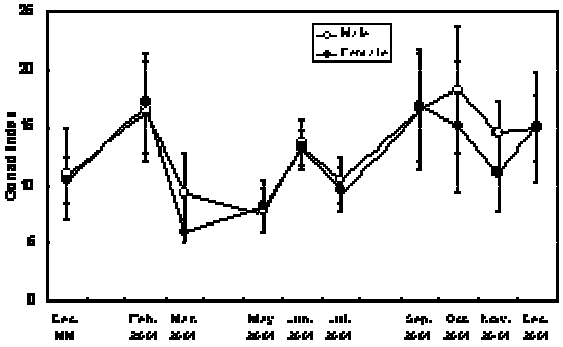

Figure 4. Seasonal changes in the GI of male and female individuals

Table 2 shows the frequency distribution of the maturational stages of gonads in each month sampled. In 2001, all the individuals were in mature (stage 4) in February, while greater part of individuals had spent gonads (stage 5) in March. From May to September, all the individuals with one exception in May had immature gonads (stage 1).

Table 2. Frequency distribution of the maturational stages of gonads

Date	Total Number	Maturational stages of gonads									
		Male					Female				
		1	2	3	4	5	1	2	3	4	5
Dec. 20, MM	13				11					2	
Feb. 16, 01	25				10					15	
Mar. 27, 01	23				4	11				1	7
May 30, 01	30	17				1	12				
Jun. 26, 01	20	8					12				
Jul. 25, 01	25	13					12				
Sep. 18, 01	10	4					6				
Oct. 25, 01	24	1	9	5				9			
Nov. 19, 01	19			6	1			5	7		
Dec. 25, 01	20				12					8	

Many individuals were in stage 2 (growing) in October and in stage 3 (pre-mature) in November. All the individuals were mature (stage 4) in December. These results in the maturational stages, taken together with the changes in GI, suggest that *H. pulcherrimus* in Mikuni initiates gametogenesis around October and spawns around February to March.

Changes in the Pul content in ovaries are shown in Fig. 5. The mean Pul level steadily decreased from December 2000 (2.6 mg/100g) to July 2001 (0 mg/100g), and kept this value until September 2001. The values started to increase from October 2001 and reached the maximum in December 2001 (3.5 mg/100g).

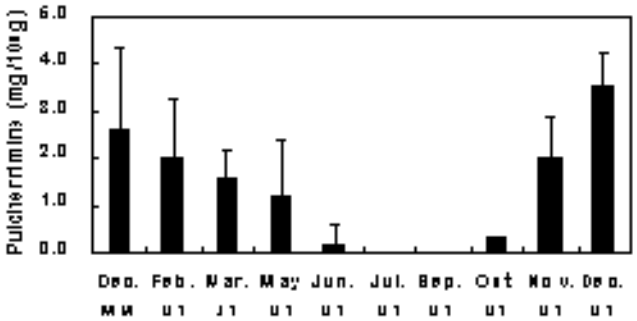

Figure 5. Changes in the Pul content in ovaries

The increase in the Pul level from September to December was in accordance with the progress of the maturational stages of ovaries shown in Table 2, indicating that Pul accumulation is related to oogenesis. Pul possibly accumulates in the oocyte or ovum as oogenesis proceeds. After the spawning season, the Pul content did not decrease rapidly. In sea urchins, residual gametes are phagocytized by NPs after spawning (Masuda & Dan 1977; Tominaga & Takashima 1987). Pul detected in the immature ovaries in May and June could be derived from the phagocytized residual ova. Probably, this Pul was degraded as the phagocytized ova gradually de-generated.

In conclusion, Pul accumulates in the ovary of *H. pulcherrimus* as oogenesis proceeds, and gradually disappears after spawning.

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