KOREAN JOURNAL OF ZOOLOGY
Vol. 29, No. 3, pp. 190-200 (1986)

Protein Patterns of *Cipangopaludina chinensis malleata* (Reeve) by SDS/PAGE and Amino Acid Analysis

Won Chul Choi
(Department of Biology, College of Natural Sciences, Pusan National University)

SDS/PAGE와 아미노산분석에 의한 논우렁이[*Cipangopaludina chinensis malleata* (Reeve)]의 단백질 패턴

최 원 철
(부산대 자연대 생물학과)

(Received February 27, 1986)

요 약

논우렁이의 組織別 蛋白質 패턴에 있어서 筋肉은 雌雄에 關係없이 16個의 主要蛋白質 bands는 거의 同一 하였으며, 그 중에서 bands E(Mr. 41,500), H(Mr. 52,100), L(Mr. 71,700), N(Mr. 98,500), O(Mr. 107,900)와 P(Mr. 112,900)는 筋肉과 精巢및 卵巢에서 나타나는 蛋白質이었다. 그러나 精巢나 卵巢의 蛋白質 패턴은 그들 固有의 蛋白質 패턴을 가지고 있었으며 組織에서 全部 나타나는 bands外에 精巢와 卵巢에서 共同으로 나타나는 主要한 bands는 약 5個[bands d(Mr. 15,600), k(Mr. 37,100), p(Mr. 57,000), s(Mr. 80,300), v(Mr. 105,400)]로 나타남을 알수 있었다.

그리고 主要蛋白質 bands를 elution시켜서 아미노산을 分析한 結果는 단백질 패턴의 양상과 같이, 아미노산의 量과 組成이 많은 순서는 卵巢, 筋肉, 精巢의 順으로 되었다. 이러한 結果를 볼때 卵巢에서는 全個體에서 가질수 있는 豊富한 蛋白質이 發見되는 同時에 精巢에서는 核酸物質의 濃縮으로 精蟲이 되기 위한 morphogenesis의 現象으로 말미암아 蛋白質의 量이 매우 적게 나타나는 것으로 生覺되었다.

INTRODUCTION

Many studies have been made on the analysis of protein patterns and amino acid composition of Bivalvia of Mollusca (Hujita *et al.*, 1968; Ito, 1959; Konosu and Mori, 1959). In Gastropoda analysis of amino acid of Korean slug and land snail has been studied by Kim *et al.* (1983), and the protein pattern of Bivalvia by SDS/PAGE has been reported

by Choi and Ha (1985). But there have been few reports dealing with the protein pattern and amino acid analysis in ***Cipangopaludina chinensis malleata.***

In this study, it is attempted to analyze the amino acids in the characteristic protein bands in order to obtain the essential data on the protein composition of each tissue of the mud-snail by SDS/PAGE. The molecular weights of protein of tissues are estimated by the personal computer.

MATERIALS AND METHODS

Adult mud-snails, *Cipangopaludina chinensis malleata,* were collected for two months (August and September) at suburbs of Kyungju, Kyungpook, Korea.

For sample preparations, the muscles, testes and ovaries of animals were dissected out under the stereomicroscope (Olympus, Japan). Each tissue was diluted ten times with 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% SDS (sodium dodecyl sulfate), 5% 2-mercaptoethanol and 10% sucrose. They were ground with sea sand in mortar dishes. The brei were filtered through Whatman No. 1 filter paper and dialyzed against 62.5 mM Tris-HCl (pH6.8) by a dialysis membrane (250-7U, Sigma) at 4°C. One volume of the dialyzed protein was added to the 9 volume of the Tris-HCl buffer (pH6.8). The mixture was heated to 100°C for 2 min and then cooled. A small amount of sucrose was added to the mixture and bromophenol blue was used as a marker.

The SDS/polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970), in which resolving and stacking gels contained 10% and 3.5% acrylamide, respectively. Twenty μl each of samples was layered on the sample slots of the stacking gel. Electrophoresis was performed at 100V for 5 hours at 4°C by using the vertical slab gel kit (Manhattan, Korea).

The electrophoresed gels were stained with 0.1% Coomassie brilliant blue R250 in a mixed solution of 45% methanol and 10% acetic acid for 2 hours at 37°C, and destained with a mixed solution of 12.5% isopropanol and 10% acetic acid for 24 hours by replacing every 8 hours with a fresh destaining solution. They were fixed in a 7% acetic acid solution and scanned with a DMU-330 densitometer (Toyo, Japan) at 570 nm.

For the estimation of molecular weights, lysozyme (Mr. 14,300), β-lactoglobulin (Mr. 18,400), trypsinogen (Mr. 24,000), egg albumin (Mr. 45,000) and bovine albumin (Mr. 66,000) (Sigma) were used as marker protein.

On the basis of the methods of Peacock and Dingman (1968), Loening (1968), Swank and Munkers (1971), and Neville (1971), the estimation of molecular weights was modified to be suited for computer program. A personal computer system which was included keyboard (Apple II Comote DAEAM, Korea) and printer (SMD SX-85 CA, Japaan) was used.

For the amino acid analysis of the charateristic bands after SDS/PAGE, the proteins of the characteristic bands were eluted using the modified disc-electrophoresis kit. The gel

slices containing the band of interest were put into the long glass tubes (30×0.5 cm). One end of the tube was sealed with parafilm. The stacking gel was then poured into the tube. Every slice and stacking gel in the tube were allowed to be embedded and polymerized. After polymerizaton, the parafilm was removed and a small dialysis membrane-sack made and filled with electrophoretic buffer was fastened with rubber bands over the end of the glass tube. Running was conducted at 100V for 24 hours at 4°C. The eluted proteins within the membrane sack were obtained using a syringe. A new glass tube (60×0.5 cm) was prepared and sealed at one end of the glass tube by heating. The sample of 5 ml was injected to the glass tube after being diluted 2 times with 6 N HCl. The mixed sample was

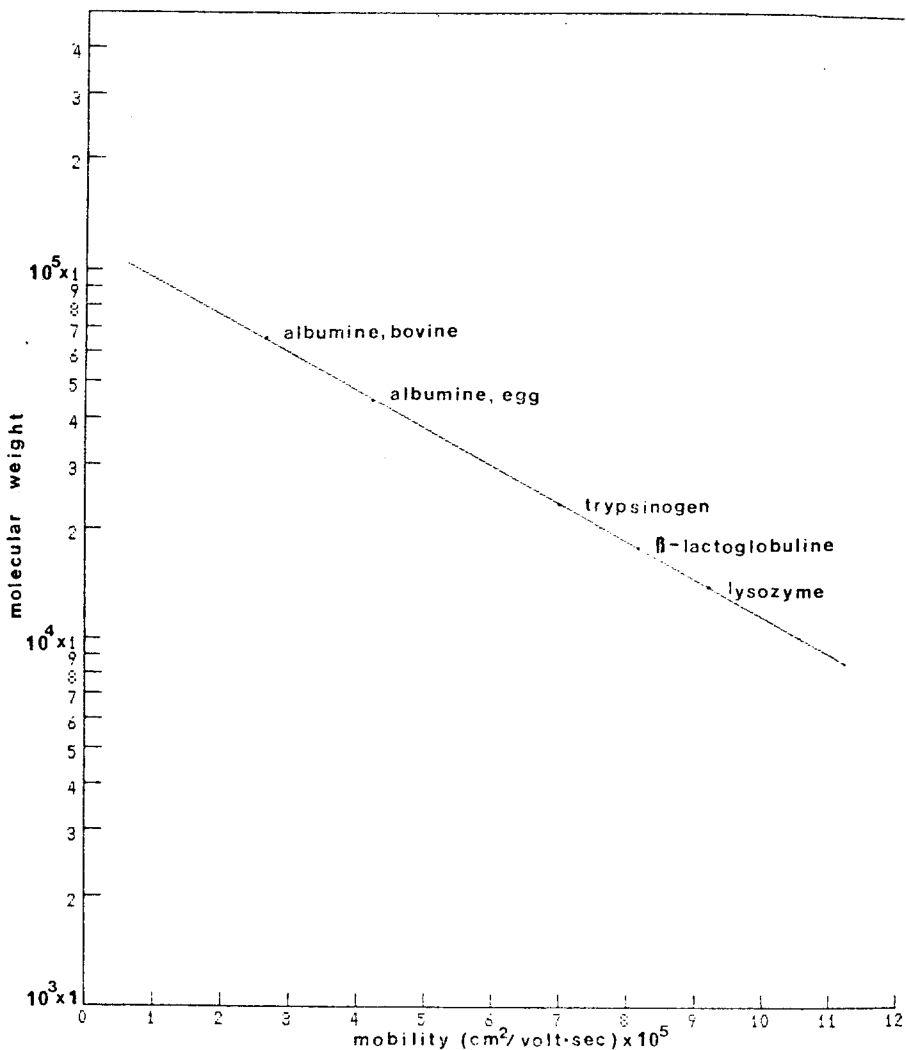

Fig. 1. Relation between the molecular weight of marker proteins and their mobilities.

degassed for 90 sec using a vacuum pump (Wyzenbeek and Staff, Chicago, U.S.A.), and then the other end of the glass tube was sealed by heating. The sample in the glass tube was hydrolyzed for 24 hours at 110°C. Amino acid was analyzed with an high-speed automatic amino acid analyzer (Hitachi, Model 835-50, Japan).

RESULTS

The estimation of molecular weight of each band became possible by the use of the computer program.

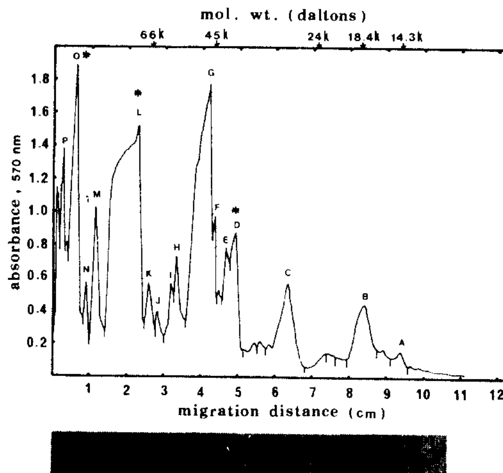

Fig. 2. Protein pattern of adult female muscle. alphabetical order; protein bands. *: amino acid-analyzed band.

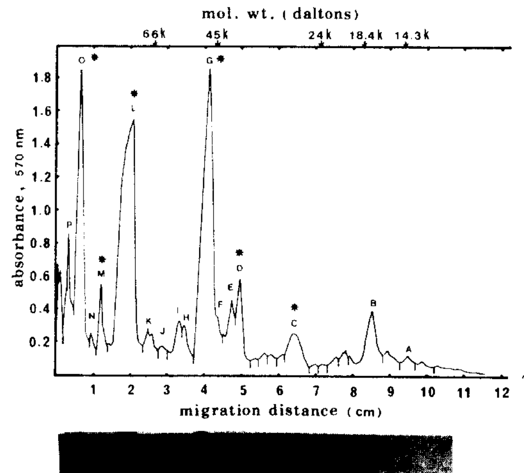

Fig. 3. Protein pattern of adult male muscle. alphabetical order: protein bands. *: amino acid-analyzed band.

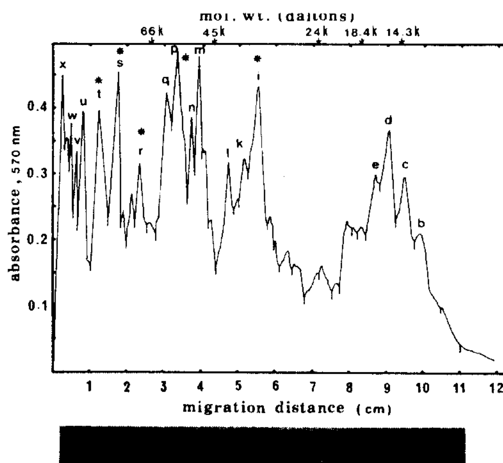

Fig. 4. Protein pattern of testis. alphabetical order: protein bands. *: amino acid-analyzed band.

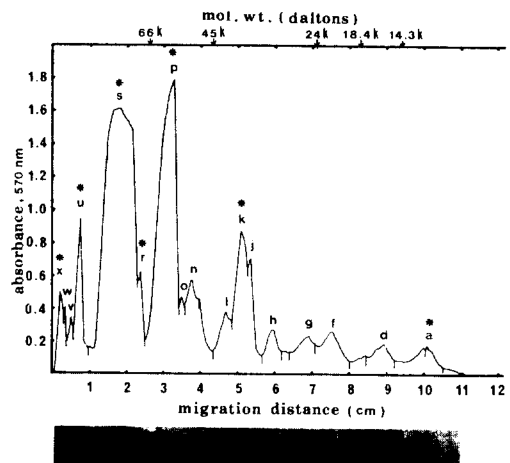

Fig. 5. Protein pattern of ovary containing early embryos. alphabetical order: protein bands. *: amino acid-analyzed band.

The protein patterns of the muscles and testis are shown in Figs. 2~4, and that of ovary which contained an embryo growing before the development of the shell after fertilization, is shown in Fig. 5.

The protein patterns of both the male and female muscles were fairly similar. Particularly the intensity of the band F (Mr. 44,500) appeared strongly in the female than in the male (Figs. 1 and 2). The larger quantity of proteins contained in the male and female muslces was found in bands L (Mr. 71,700), G (Mr. 46,500) and 0 (Mr. 107,900), and the intensity as shown by the absorbance 750 nm decreased in the order of the bands 0, G and L. The protein bands in the area of over. 39,700 dalton was denser than those of low molecular weight.

Table 1 shows the result of the amino acid composition of bands D (Mr. 39,700), L (Mr. 71,700) and 0 (Mr. 107,900) that are marked with asterisk in the protein bands of the female muscles.

Table 2 shows the amino acid composition of the bands C(Mr. 28,900), D(Mr. 39,700), G(Mr. 46,500), L(Mr. 71,700), M(Mr. 94,100) and 0(Mr. 107,900). These bands are marked with asterisk in the protein bands (Fig. 3) of male muscles.

The protein pattern of the testis did not show any characteristic bands and the quantity of proteins was not as large as the other tissues such as that of muscle (Figs. 1 and 2) and of ovary (Fig. 5), but protein bands were distributed rather evenly in all the ranges of molecular weights (Fig. 4). In addition, the intensity of the protein in the testis was generally weaker than those of the other tissues. The result of the amino acid analysis of eluted protein bands i (Mr. 34,600), p (Mr. 57,000), r (Mr. 71,700), s (Mr. 80,300) and t (Mr. 82,100) is shown in the Table 3.

The quantity of proteins in the ovary containing the embryo and oocyte before the dev-

Table 1. Amino acid contents of the asterisk-marked bands in Fig. 2.

amino acids	D	L	O
	means±S.D.	means±S.D.	means±S.D.
glutamic acid	—	0.222±0.015	—
glycine	—	—	0.118±0.010
cysteine	1.427±0.084	0.867±0.049	—
valine	0.045±0.002	0.045±0.003	1.082±0.098
isoleucine	0.060±0.002	0.059±0.004	0.076±0.006
leucine	0.074±0.006	0.078±0.101	0.150±0.017
phenylalanine	1.579±0.281	1.894±0.006	1.496±0.170
lysine	0.084±0.007	0.079	0.125±0.009
arginine	—	—	0.081±0.007
proline	—	0.091±0.009	—

S.D.; standard deviations. Means indicate mg% of amino acids.

Table 2. Amino acid contents of the asterisk-marked bands in Fig. 3.

amino acids	C	D	G	L	M	O
	means±S.D.	means±S.D.	means±S.D.	means±S.D.	means±S.D.	means±S.D.
aspartic acid	0.313±0.047	—	—	—	—	—
threonine	0.060±0.007	—	—	—	—	0.087±0.009
serine	—	0.223±0.038	0.081±0.009	—	0.088±0.009	0.029±0.004
glutamic acid	0.226±0.038	0.307±0.050	0.069±0.007	—	—	0.145±0.023
alanine	—	0.383±0.058	0.269±0.042	—	0.238±0.038	—
cysteine	0.048±0.006	0.718±0.091	0.528±0.078	—	0.498±0.067	0.524±0.061
valine	0.039±0.005	0.053±0.006	0.037±0.004	1.203±0.222	0.031±0.004	0.035±0.004
isoleucine	0.044±0.006	0.060±0.005	0.042±0.005	0.080±0.011	0.035±0.004	0.034±0.004
leucine	0.041±0.006	0.064±0.006	0.054±0.006	0.104±0.024	0.041±0.006	0.049±0.003
phenylalanine	2.910±0.408	2.306±0.309	1.389±0.219	2.088±0.309	1.457±0.209	1.336±0.209
lysine	0.103±0.009	0.096±0.011	0.063±0.008	0.111±0.008	0.088±0.010	0.055±0.006
histidine	—	—	0.033±0.005	—	—	—
arginine	—	—	—	0.042±0.006	—	—

S.D.; standard deviations, Means indicate mg% of amino acids.

Table 3. Amino acid contents of the asterisk-marked bands in Fig. 4.

amino acids	i	p	r	s	t
	means±S.D.	means±S.D.	means±S.D.	means±S.D.	means±S.D.
aspartic acid	0.037±0.003	—	—	0.035±0.004	0.055±0.006
threonine	0.082±0.005	—	—	—	0.097±0.016
serine	0.031±0.004	0.201±0.019	0.078±0.009	0.125±0.027	0.028±0.001
glutamic acid	0.118±0.020	0.037±0.004	0.061±0.005	0.040±0.005	0.100±0.009
glycine	—	—	—	0.162±0.027	—
alanine	—	0.426±0.041	0.265±0.037	0.345±0.048	—
cysteine	—	0.943±0.108	0.602±0.087	0.059±0.007	0.056±0.007
valine	—	0.046±0.005	0.037±0.002	0.054±0.006	—
isoleucine	0.035±0.004	0.053±0.004	0.049±0.005	—	0.035±0.004
leucine	0.035±0.003	0.066±0.007	0.053±0.006	0.044±0.005	0.034±0.002
phenylalanine	1.709±0.209	2.488±0.376	1.293±0.197	0.701±0.091	1.913±0.265
lysine	0.059±0.007	0.097±0.008	0.050±0.004	0.048±0.005	0.070±0.006

S.D.; standard devitaions, Means indicate mg% of amino acids.

elopment of the shell increased in the order of the bands s, p and k... etc. The intensities of the bands appeared in the order of p, s, u, and k... etc. (Fig. 5). Protein bands marked with asterisk (Fig. 5), including a (Mr 12,200), K (Mr. 37,100), p (Mr. 57,000), r (Mr. 71,700), s (Mr. 80,300), u (Mr. 98,500) and x (Mr. 112,900), were eluted from the gel band and their composition of amino acid was analyzed (Table 4).

Table 4. Amino acid contents of the asterisk-marked bands in Fig. 5.

amino acids	a	k	p	r	s	u	x
	means±S.D.	means±S.D.	means±S.D.	means±S.D.	means±S.D.	means±S.D.	means±S.D.
aspartic aicd	—	0. 299±0. 041	—	—	0. 232±0. 031	—	—
threonine	—	0. 108±0. 021	0. 038±0. 004	—	0. 034±0. 004	—	—
serine	—	0. 452±0. 053	0. 089±0. 012	0. 280±0. 004	—	—	—
glutamic acid	—	0. 102±0. 009	0. 050±0. 007	0. 364±0. 041	—	0. 323±0. 044	0. 180±0. 008
glycine	—	0. 262±0. 031	0. 128±0. 021	—	—	—	—
alanine	—	0. 606±0. 076	0. 368±0. 054	0. 361±0. 044	—	—	—
cysteine	—	0. 055±0. 006	0. 059±0. 006	0. 969±0. 119	0. 052±0. 006	1. 090±0. 228	0. 487±0. 031
valine	1. 297±0. 230	0. 088±0. 009	0. 051±0. 004	0. 078±0. 009	0. 040±0. 005	0. 045±0. 007	0. 042±0. 003
methionine	—	0. 031±0. 003	—	0. 037±0. 002	—	—	—
isoleucine	0. 062±0. 007	0. 067±0. 008	0. 052±0. 006	0. 065±0. 007	—	0. 060±0. 004	0. 055±0. 004
leucine	0. 056±0. 006	0. 043±0. 005	0. 044±0. 005	0. 066±0. 007	0. 032±0. 005	0. 052±0. 007	0. 053±0. 003
phenylalanine	2. 147±0. 327	3. 386±0. 428	2. 368±0. 329	2. 474±0. 333	1. 108±0. 205	2. 748±0. 022	1. 482±0. 098
lysine	0. 090±0. 008	0. 080±0. 009	0. 167±0. 008	0. 134±0. 021	0. 072±0. 009	0. 084±0. 006	0. 050±0. 004
histidine	—	0. 073±0. 008	—	0. 040±0. 006	—	—	0. 032±0. 002
arginine	—	—	0. 049±0. 006	—	—	—	—
proline	—	—	—	—	—	0. 100±0. 008	0. 080±0. 006

S.D.; standard deviations, Means indicate mg% of amino acids.

Table 5. Protein patterns of *Cipangopaludina chinensis malleata.*

M.W. \ tissue	female muscle	male muscle	testis	ovary (containing embryos)
12, 200				a
13, 000			b	
14, 000	←········	A ········→	←···· c ····→	
15, 600			←········	d ········→
16, 700			e	
17, 900	←········	B ········→		
22, 000				f
25, 200				g
28, 900	←········	C ········→		
31, 600				h
34, 600			i	
36, 200				j
37, 100			←········	k ········→
39, 700	←········	D ········→		
41, 500	←————	E ————→	←————	l ————→
45, 500	←········	F ········→		
46, 500	←········	G ········→		
49, 800			m	
52, 100	←————	H ————→	←————	n ————→
54, 600				o
57, 000			←········	p ········→
59, 700	←········	I ········→	←···· q ····→	
64, 000	←········	J ········→		
68, 500	←········	K ········→		
71, 700	←————	L ————→	←————	r ————→
80, 300			←········	s ········→
82, 100			t	
94, 100	←········	M ········→		
98, 500	←————	N ————→	←————	u ————→
105, 400			←········	v ········→
107, 900	←————	O ————→	←————	w ————→
112, 900	←————	P ————→	←————	x ————→

alphabetical orders: bands of tissues (see Figs. 2-5)
←——→ : the band which appears in all tissues;
←······→ : the band which appears in some tissues;
M.W.: molecular weight (daltons), rounding off to the nearest 100

When the occurrence of protein bands in each tissue is compared, the bands b, e, i, m, and t occurred only in the testis and those in the ovary only were a, f, g, h, j, and o. Those in both organs were the bands d (Mr. 15, 600, k (Mr. 37, 1000), l (Mr. 41, 5000), n (Mr. 52, 100), p (Mr. 57, 000), r (Mr. 71, 700), s (Mr. 80, 300), u (Mr. 98, 500), v (Mr. 105, 400), w (Mr. 107, 900) and x (Mr. 112, 900). Table 5 compares the occurrence of

the protein bands in various organs tested. But the molecular weight of the band l equals to that of the band 'E in the male and female muscle. That of the band n to that of the band H of the muscles. That of the band r to that of the band L of the muscles, and that of the band u to that of the band N of the band N of the muscles. Those of the bands w and x equal to those of the bands O and P of the muscles, respectively. While the bands of c and q in the testis did not appear in the ovary, they had the same molecular weights as those of A and I in the muscles, respectively.

DISCUSSION

The protein patterns in various tissues of adult *Cipangopaludina chinensis malleata* show slight difference from easch other, but the pattern and quantity of protein were similar in the muscles. The results are in good agreement with that of Choi and Ha (1985). In their study, *Scapharca subcrenata* and *Scapharca broughtonii* showed similar appearance in the protein patterns of the muscles. Thus, on the basis of these experimental results, it appears to be hard to identify the sexes by analyzing the proteins of the muscles with the method of SDS/PAGE. However, when randomly selected protein bands are eluted for the analysis of amino acid, it is interesting that even the bands with the same molecular weight could have different compositions of amino acids as revealed by the repeated experiments. This proves that even the protein with the same molecular weight could have different compositions of amino acids. Therefore, it could be emphasized that studies on the sequence of amino acids should be done within the framework of the analysis of the composition of protein.

The testis and the ovary have their own characteristic protein patterns. The testis produces many sperms through the spermatogenesis and spermiogenesis (Choi, 1985; Kim and Choi, (1986). During the process of spermiogenesis, the cytoplasm of the spermatid contained much more ribosomes consisted in ribonucleic acid (RNA) than the protein synthesized from ribosomes and many lysosomal vacuoles underwent the exocytosis (Kim and Choi, 1986). Owing to the condensation of the nucleus and the glycogen-containing glycoprotein and glycolipid (Anderson and Personne, 1970) being so much in the tail part of sperm, it is natural that the protein in the testis was less detected than the nucleic acid.

The oocyte in the ovary generally produced much of the yolk protein, which, according to Choi and Jo (1983), was also produced not only in *Marphyra sanguinea* but also in insect oocyte (Choi, 1977). Jang and Lee (1981) reported that the synthesis of protein remarkably increased during the blastula and gastrula in sea urchin after fertilization. This reveals that protein increases in proportion to the development of the oocyte and the embryo. In the light of this, it is natural that in *Cipangopaludina chinensis malleata*, the species of ovo-viviparity, much proteins appear in the ovary containing the developing embryo before the development of the shell. The analysis of the amino acid in the characteristic bands also shows that the quantity of protein in the testis is the smallest, while the composition and that of

protein in the ovary is the largest. Thus, the protein pattern of this species has its own characteristic band, and it is shown that there appear more variation in the order of the testis, the ovary, and the muscles, and quantitative compositions in the order of the ovary, the muscles, and the testis.

Because the personal computer easily performs the estimation of the molecular weights, in the future study the molecular weight of the characteristic protein band in the different tissues should be compared with each other. Furthermore, the composition and the sequences of amino acids which are contained in the protein with the same molecular weights should be studied and compared with one another. The difference in the development of this species according to the stage of growth and the terms divided by season should be studied more.

ABSTRACT

The male and female muscle of the 16 major bands in the protein patterns of the tissues of the mud-snail, ***Cipangopaludina chinensis malleata***, were almost similar, and the bands E (Mr. 41, 500), H (Mr. 52, 100), L (Mr. 71, 700), N (Mr. 98, 500), O (Mr. 107, 900) and P (Mr. 112, 900) had the same molecular weights which appear in the muscles, testis and ovary. But it showed that the testis and the ovary had their characteristic patterns, and it was also observed that, besides the bands common to all tissues, the bands which appear both in the testis and the ovary are about five: the bands d (Mr. 15, 600), k (Mr. 37, 100), p (Mr. 57, 000), s (Mr. 80, 300), and v (Mr. 105, 400).

The result of the amino acid analysis showed that the quantity and composition of amino acid appeared, as the protein patterns did, in the order of ovary, muscles and testis. This reveals that in the ovary a great quantity of protein which appears in the whole body was investigated, while the testis contained only little protein due to the phenomenon of morphogenesis in which sperms were developed through the condensation of the nuclei.

REFERENCES

Anderson, W.A. and D. Daul, 1976. The molluscan spermatozoon: Dynamic aspects of its structure and function. *Amer. Zool.* **16**:293-313.

Choi, W.C., 1977. Einige ultrastruktulle Aspekte der Dotterbildung der telotrophischen Ovarien bei *Gerris najas* DEG. *Theses Coll. Korea Soc. Work Coll.* 7:227-238.

Choi, W.C., 1985. The spermiogenesis of *Cipangopaludina chinensis malleata*(Reeve). *Korean J. Lim.* **18**:23-33.

Choi, W.C. and M.J. Ha, 1985. The comparison of protein patterns of several species in Bivalvia by SDS/polyacrylamide gel electrophoresis. *Korean J. Zool.* 28:61-70.

Choi, W.C. and U.B. Jo., 1983. Histological study on the oogenesis of *Marphysa sanguinea*. *J. Sci. (BNU)* 35:183-193.

Hujita, M., Yeh, S-j. and Ikeda, S., 1968. Studies on chemical components of Japanese pearl-oyster

meat-I. Constituents of the extracts of adductor muscles. *Bull. Japanese Soc. Sci. Fish.* **34**:146-149.

Ito, K., 1959. Amino acid composition of the muscle extracts of aquatic animals-Ⅱ. The amounts of free amino acids in the muscle of shellfishes and their variation during spoilage. *Bull. Japanese Soc. Sci. Fish.* **25**:658-660.

Jang, J.W. and Y.R. Lee, 1985. Changes in the RNA and protein synthesis at the pre- and post-fetilization stages of a sea urchin, *Hemicentrotus pulcherrimus. Korean J. Zool.* **28**:71-84.

Kim, J.H. and W.C. Choi, 1986. Electron microscopic study on the spermiogenesis of *Cipangopaludina chinensis malleata* (Reeve). *Korean. J. Zool.* **29**:121-140.

Kim, S. -K., S. -H. Moon, E. -H. Lee and O. -J. Kang, 1983. Studies on the chemical compostion of Korean slug, *Incilaria frusorferi*, and Korean land snail, *Acusta despecta* (GRAY). (1) Mineral content and amino acid composition of Korean slug and Korean land snail. *Bull. Nat. Fish. Univ. Busan* **23**:15-21.

Konosu, S. and T. Mori, 1959. Amino acid composition of shellfish proteins. *Bull. Japanese Soc. Sci. Fish.* **25**:153-155.

Laemmli, U.K., 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.

Loening, U., 1968. Molecular weights of ribosomal RNA in relation to evolution. *J. Mol. Biol.* **38**: 355-365.

Neville, D.M., Jr., 1971. Molecular weight determination of protein-dodecyl sulfate discontinuous buffer system. *J. Biol. Chem.* **246**:6328-6334.

Peacock, A.C. and C.W. Dingman, 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose acrylamide composite gels. *Biochem.* **7**:668-674.

Swank, R.T. and K.D. Munkres, 1971. Molecular weight analysis of oligopeptides by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. *Anal. Biochem.* **39**:462-477.