



## **Purification and Properties of Androgenic Gland Hormone from the Terrestrial Isopod *Armadillidium vulgare***

Authors: Okuno, Atsuro, Hasegawa, Yuriko, and Nagasawa, Hiromichi

Source: Zoological Science, 14(5) : 837-842

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.14.837>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Purification and Properties of Androgenic Gland Hormone from the Terrestrial Isopod *Armadillidium vulgare*

Atsuro Okuno<sup>1,3</sup>, Yuriko Hasegawa<sup>2</sup> and Hiromichi Nagasawa<sup>1,3\*</sup>

<sup>1</sup>Ocean Research Institute, The University of Tokyo, Nakano-ku, Tokyo 164, Japan

<sup>2</sup>Department of Biology, Keio University, Hiyoshi, Yokohama 223, Japan

---

**ABSTRACT**—The androgenic gland hormone (AGH) is known to control sex differentiation in crustaceans. AGH was purified from isolated androgenic glands (AGs) of the male isopod *Armadillidium vulgare* by three steps of reverse-phase high performance liquid chromatography (RP-HPLC) and its chemical properties were examined. The purified AGH-active fraction showed masculinizing activity when 38 pg of this preparation was injected into a young female of the same species. Only 160 ng of the material was obtainable from 2000 animals at about an 11% rate of recovery. The elution of AGH activity by molecular sieve HPLC indicated that molecular weight of AGH was 11,000 ~ 13,000. AGH was inactivated by treatment with trypsin or by reductive carboxymethylation. The AGH activity was not affected by heat treatment at 100°C for 3 min. These results indicated that AGH was a heat-stable protein with disulfide bond(s).

---

## INTRODUCTION

Male sexual differentiation in malacostracan crustaceans is governed principally by androgenic gland hormone (AGH) secreted from the androgenic gland (AG) (reviewed by Katakura, 1984; Charniaux-Cotton and Payen, 1985). In order to elucidate the chemical nature of AGH, some investigators have attempted to isolate AGH and to perform inactivation experiments. Previously, two lipophilic compounds, farnesylacetone and hexahydrofarnesylacetone, extracted from hemolymph and AGs of the shore crab *Carcinus maenas* (Berreur-Bonnenfant *et al.*, 1973; Férézou *et al.*, 1977) were considered as candidates for AGH, but neither of them demonstrated masculinizing activity (Charniaux-Cotton, 1972; Juchault *et al.*, 1984). Other investigations encompassing ultrastructural and histological studies suggested that AGH was a protein (King, 1964; Meusy, 1965; Malo and Juchault, 1970; Chaigneau and Juchault, 1979). Along these lines, water-soluble, proteinaceous material showing masculinizing activity was extracted from the isopod *Armadillidium vulgare* by two research groups (Katakura *et al.*, 1975; Juchault *et al.*, 1978, 1984). However, there were discrepancies in heat-stability and molecular weight (MW) of AGH estimated by two groups; heat-unstable nature and MW of 15,000 ~ 17,000 (Katakura *et al.*, 1975) vs. heat-stable nature and MW of 2,000 ~ 8,000 (Juchault *et al.*, 1978, 1984).

AGH was purified from whole male reproductive organs

including the AGs of *A. vulgare* by an 8-step procedure to obtain two active fractions termed AGH I and AGH II, and their chemical properties were clarified (Hasegawa *et al.*, 1987). Later, the AGH I fraction was found to comprise mainly four structurally-related peptides, but Western blotting analysis using an antiserum raised against these peptides showed their presence only in the seminal vesicle and the vas deferens, indicating that AGH was a minor component of the AGH I fraction (Nagasawa *et al.*, 1995). Independent study on purification of AGH from the hypertrophied AGs of intersexed animals of *A. vulgare* succeeded in obtaining two active fractions, whose MWs were both estimated to be 17,000 ~ 18,000 (Martin *et al.*, 1990). Amino acid analyses of these two fractions indicated the absence of cysteine and considerable differences in composition, suggesting that at least one of them was still impure.

In this paper, we describe reexamination of chemical properties and MW of AGH from normal males and purification of AGH from the AGs of normal male animals of *A. vulgare* by three steps of reverse-phase high performance liquid chromatography (RP-HPLC).

## MATERIALS AND METHODS

### Animals

Males and females of the terrestrial isopod *Armadillidium vulgare* were gathered from shrubbery around the campuses of the Ocean Research Institute in Nakano, Tokyo, and of Keio University, Yokohama, Japan. Three pairs of AG-testis complex of a mature male of *A. vulgare* larger than 9 mm in body length, were extirpated from the whole reproductive organ. These were immediately placed on a slide glass and dried in a vacuum-desiccator. The androgenic glands only were removed from the testes with a razor blade under a microscope and kept in a 1.5-ml polypropylene microtube. For some inac-

---

\* Corresponding author: Tel. +81-3-3812-2111 ext. 5132;  
FAX. +81-3-5802-2932.

<sup>3</sup> Present address: Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

tivation experiments, AG-testis complexes were collected in woodlouse Ringer solution (Sutton, 1972). They were stored at  $-30^{\circ}\text{C}$  until extraction.

### Bioassay of AGH

Bioassay of AGH was performed essentially according to methods reported previously (Hasegawa *et al.*, 1987). Samples to be assayed were added with 5  $\mu\text{g}$  of BSA in an aqueous solution, dried by vacuum centrifuge and then dissolved in Ringer solution. The addition of BSA helped to minimize losses prior to assay. Ten young *Armadillidium* females, 6–7 mm in body length and 10–20 mg in body weight, received a single injection of 0.5  $\mu\text{l}$  of serially-diluted sample solution. After the next molt, usually from 5 to 14 days later, they were examined under a dissecting microscope for elongation of the endopodites of the first pair of abdominal legs, the criterion for masculinization. In this assay, false positive responses were very rare. Therefore, we regarded the samples as active when even a low percentage of injected females showed positive response. One *Armadillidium* unit (unit) was defined as the minimum amount necessary to cause masculinization in more than a half of the injected females (usually  $n=10$  at each dose).

### Purification of AGH

The androgenic glands from 2000 animals were homogenized on ice in 0.5 ml of 20 mM Tris-HCl buffer (pH 8.0) containing 0.5 mM phenylmethanesulfonyl fluoride (Wako Pure Chemicals) using a Polytron homogenizer (Kinematica AG, Switzerland), and the homogenate was kept on ice for 3 min. After centrifugation at  $17,500 \times g$  and  $4^{\circ}\text{C}$  for 10 min, the pellet was homogenized and centrifuged in the same manner as above. The two supernatants were combined, adjusted to pH 2.0 by the addition of 5  $\mu\text{l}$  of 10% trifluoroacetic acid (TFA), and centrifuged at  $17,500 \times g$  and  $4^{\circ}\text{C}$  for 10 min.

The supernatant was then subjected to the first RP-HPLC using an Asahi Pak ODP-50 column ( $4.6 \times 150$  mm, Showa Denko, Tokyo). Separation was performed with a 21.33-min linear gradient of 8–40% acetonitrile in 0.05% TFA at a flow rate of 1 ml/min. Each fraction was collected manually every minute and AGH activity was measured by an *in vivo* bioassay. Elution was monitored by measuring the absorbances at 225 and 280 nm and the amount of protein in the active fraction was measured by using bovine serum albumin (BSA) as a standard. The concentrated active fraction from the first HPLC was further purified by the second RP-HPLC using a  $\mu$ -Bondasphere column ( $3.9 \times 150$  mm, 5  $\mu$  phenyl 300 Å, Waters) with a 32-min linear gradient of 0–32% acetonitrile in 0.05% TFA at a flow rate of 1 ml/min. Fractions were collected manually every minute, and the elution was monitored by the absorbances at 225 and 280 nm. An aliquot of each fraction was subjected to bioassay. The active fraction from the second HPLC was concentrated and chromatographed by the third RP-HPLC using the same column as in the first HPLC with a 40-min linear gradient of 20–40% acetonitrile in 0.05% heptafluorobutyric acid (HFBA) at a flow rate of 1 ml/min. All peak materials were collected manually by monitoring the absorbances at 225 and 280 nm, and the activity of each peak material was measured by using a bioassay.

### Heat treatment

Each of the AGH active fraction (750 units) obtained by RP-HPLC of AG extract, and crude AG-testis extract (1500 units) was divided equally into three parts for control, acidic condition and neutral condition. For acidic condition, the samples were heated at  $100^{\circ}\text{C}$  for 3 min in 0.05% TFA. For neutral condition, the AGH fraction and AG-testis extract were heated at  $100^{\circ}\text{C}$  for 3 min in 20 mM Tris-HCl buffer (pH 8.0) and in Ringer solution for AG-testis extract, respectively. The controls were not heated. The controls and the samples after treatment under neutral conditions were adjusted to 0.05% TFA by the addition of 10% TFA. All the samples were centrifuged at  $17,500 \times g$  and  $4^{\circ}\text{C}$  for 10 min. Each supernatant was concentrated and examined for biological activity.

### Trypsin digestion

The AGH active fraction (30 units), which was obtained by precipitation with 80% saturation of ammonium sulfate and two steps of RP-HPLC from AG-testis extract, was dissolved in 30  $\mu\text{l}$  of 0.1 M Tris-HCl buffer (pH 9.0), to which 1  $\mu\text{g}$  of TPCK-treated trypsin (Sigma) in 1 mM HCl (1 mg/ml) was added. The mixture was maintained at  $37^{\circ}\text{C}$  for 3 hr with occasional shaking. The digestion was stopped by the addition of 10  $\mu\text{g}$  of trypsin inhibitor in distilled water (4 mg/ml, from soybean, Wako Pure Chemicals). The resulting mixture was lyophilized and assessed for biological activity.

In order to observe the specificity of the enzyme, 10  $\mu\text{g}$  of trypsin inhibitor in 30  $\mu\text{l}$  of 0.1 M Tris-HCl buffer (pH 9.0) was added to the mixture before incubation. For examination of the effects of trypsin inhibitor on AGH activity, 10  $\mu\text{g}$  of trypsin inhibitor was added to the AGH fraction in 30  $\mu\text{l}$  of 0.1 M Tris-HCl buffer (pH 9.0) without trypsin after incubation. A mixture of 1  $\mu\text{g}$  of trypsin and 10  $\mu\text{g}$  of trypsin inhibitor in 30  $\mu\text{l}$  of 0.1 M Tris-HCl buffer (pH 9.0) was incubated without the AGH fraction. The samples from all groups were examined for biological activity.

### Reductive carboxymethylation

Partially purified AGH fraction (240 units), which was obtained by precipitation with 80% saturation of ammonium sulfate and RP-HPLC from AG-testis extract, was dissolved in 20  $\mu\text{l}$  of 1M Tris-HCl buffer (pH 9.0), and added with 10  $\mu\text{l}$  of dithiothreitol solution (1 mg/ml, 0.1 M Tris-HCl buffer, pH 9.0). The resulting mixture was incubated at  $35^{\circ}\text{C}$  for 30 min. Next, 20  $\mu\text{l}$  of sodium iodoacetate solution (2 mg/ml, 0.1 M Tris-HCl buffer, pH 9.0) was added to the reaction mixture, and the incubation was continued at  $35^{\circ}\text{C}$  for 30 min. The reaction was stopped by adding 20  $\mu\text{l}$  of 1M HCl. The reaction mixture was applied to an octadecyl silyl-silica cartridge (C<sub>18</sub> Sep-Pak, Waters). After washing with 0.05% TFA, the materials were eluted with 50% acetonitrile containing 0.05% TFA, and the eluate was lyophilized and assessed for biological activity (treatment 1). As a control, the same sample was incubated without dithiothreitol or sodium iodoacetate and run through a Sep-Pak cartridge (treatment 2). Activity of the original sample was also checked (untreated).

### Estimation of MW

The MW of the AGH was estimated by molecular sieve HPLC using a column of Superdex<sup>®</sup> 75 HR 10/30 ( $10 \times 300$  mm, Pharmacia Biotech). The AGH active fraction, which was obtained by the first RP-HPLC of AG extract, and MW markers, which were insulin (5,700, Sigma), soybean trypsin inhibitor (21,000, Wako Pure Chemicals), ovalbumin (43,000, Wako Pure Chemicals) and bovine serum albumin (67,000, Sigma), were applied to the column. The elution was performed with 0.2 M  $\text{NH}_4\text{HCO}_3$  at a flow rate of 0.5 ml/min and monitored by measuring the absorbances at 225 and 280 nm. Each fraction was collected manually every minute, dried by a vacuum centrifuge and examined for biological activity.

## Results

### Dose-response relationship of AGH

Figure 1 shows a dose-response curve of crude AG extract. The lowest AGH activity was observed with 0.025 animal equivalents. The activity was enhanced with increased dosage and reached a maximum (100%) with 0.1 animal equivalents. In this case, one unit was equivalent to the amount of 0.056 animal equivalents of this preparation.

### Purification of AGH

AGs from 2000 adult males were used for purification. AGH was extracted twice with 0.5 ml of 20 mM Tris-HCl buffer.

The crude extract was then adjusted to pH 2.0 with 10% TFA. After removal of the precipitate by centrifugation, the supernatant was applied to RP-HPLC on an Asahi Pak ODP-50 column and eluted with a gradient of acetonitrile in 0.05% TFA (Fig. 2). AGH activity was eluted only between 20 and 21 min with a total activity of 12,500 units, and 12 µg protein on the basis of absorbance at 225 nm comparing with that of BSA was recovered in this fraction. The solution possessing AGH activity was concentrated and chromatographed on a Waters phenyl column (Fig. 3). AGH activity was eluted mainly between 28 and 29 min and this fraction contained 6100 units and 3.5 µg protein. In addition, very low activity was also observed in the neighboring fractions, 27–28 min and 29–30 min.

Since the total activity of these two fractions was estimated to be less than 5% of the main active fraction, only this main active fraction was subjected to the next step of purification. The concentrated active fraction was rechromatographed on an Asahi Pak ODP-50 column using a gradient of acetonitrile in 0.05% HFBA (Fig. 4). AGH activity was observed only in the shaded peak in Fig. 4. The active material from this peak possessed 4100 units and 0.16 µg protein. The results of purification of AGH are summarized in Table 1. The three steps of RP-HPLC yielded only 0.16 µg of the active fraction at a recovery rate of about 11%. A young female was masculinized by injection of 38 pg of the final preparation.

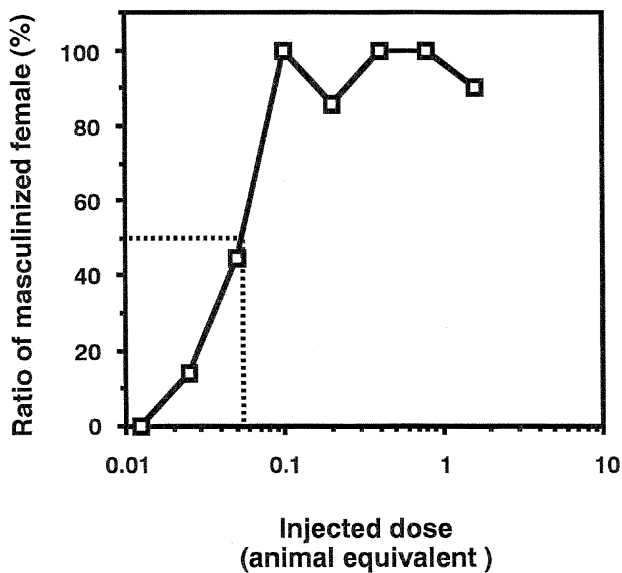


Fig. 1. Dose-response relationship of the AG extract. The dotted line indicates a 50% ratio of masculinized female. The amount of sample yielding this ratio was defined as 1 *Armadillidium* unit.

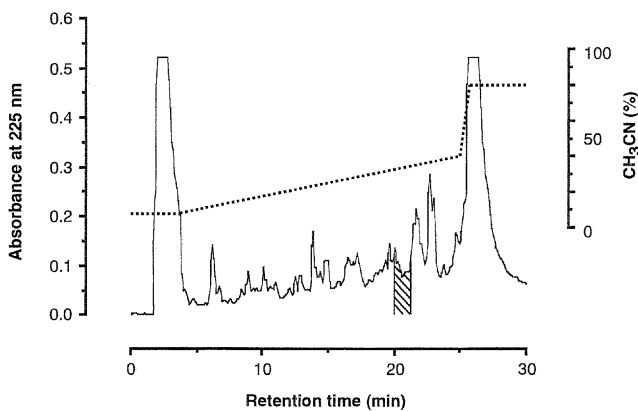


Fig. 2. Elution profile of the first RP-HPLC of AG extracts. Column: Asahi Pak ODP-50 (4.6 × 150 mm). Solvent: 8–40% acetonitrile in 0.05% TFA. Flow rate: 1 ml/min. Detection: absorbance at 225 nm. Temperature: 40°C. The concentration of acetonitrile is indicated by the dotted line. The hatched area showed AGH activity.

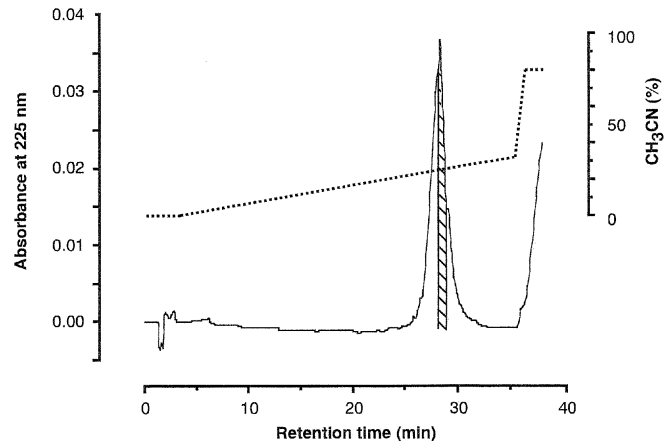


Fig. 3. Elution profile of the second RP-HPLC of active fraction from first HPLC. Column: µ-Bondasphere (3.9 × 150 mm, 5 µ phenyl 300 Å). Solvent: 16–32% acetonitrile in 0.05% TFA. Flow rate: 1 ml/min. Detection: absorbance at 225 nm. Temperature: 40°C. The concentration of acetonitrile is indicated by the dashed line. The hatched area showed AGH activity.

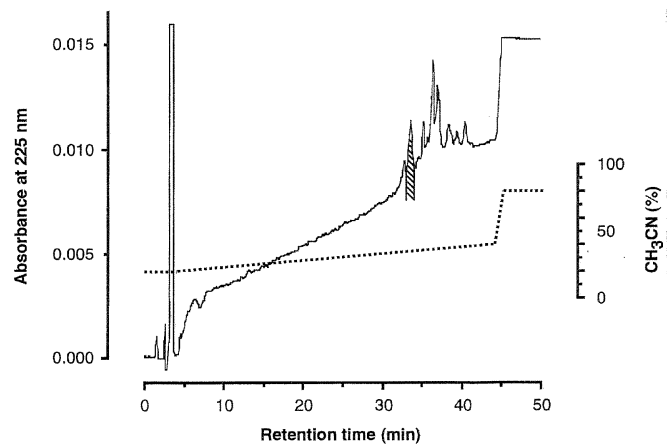


Fig. 4. Elution profile of the third RP-HPLC of active fraction from second HPLC. Column: Asahi Pak ODP-50 (4.6 × 150 mm). Solvent: 20–40% acetonitrile in 0.05% HFBA. Flow rate: 1 ml/min. Detection: absorbance at 225 nm. Temperature: 40°C. The concentration of acetonitrile is indicated by the dotted line. The hatched area showed AGH activity.

**Table 1.** Summary of purification of AGH from AGs of 2,000 animals

Purification step	Total protein (µg)	Total activity (units)	Specific activity (pg protein/unit)
Crude extract	—	36,000	—
First HPLC	12	12,500	940
Second HPLC	3.5	6,100	570
Third HPLC	0.16	4,100	38

### Chemical properties of AGH

In order to confirm the proteinaceous property of AGH, several experiments were performed. Heat-stability of AGH was examined using two different preparations, crude AG-testis extract and the AGH active fraction after purification of crude AG extract by a single RP-HPLC. Heat treatment of these two preparations gave different results. The preparation derived from only AGs was heat-stable under both neutral and acidic conditions, whereas the AG-testis extract was heat-stable under acidic conditions, but not under neutral conditions (Table 2). These results indicated that AGH itself was heat-stable and that a factor derived from the testis was responsible for heat-instability of the AG-testis extract under neutral conditions.

The AGH active fraction was digested with trypsin. Some control experiments were done in parallel, and the results are shown in Table 3. Trypsin inhibitor was used to suppress enzymatic activity of trypsin. Trypsin inactivated the AGH, but trypsin inhibitor disturbed the inactivation by trypsin only when it was added before incubation (Treatments 1 and 2). Trypsin inhibitor itself did not affect AGH activity (Treatment 3). These results indicated that AGH could be digested by trypsin, and therefore, AGH was thought to be a peptide or protein.

Next, in order to elucidate the involvement of disulfide bond(s) in AGH, the AGH active fraction was subjected to reductive carboxymethylation. This reaction brought about complete inactivation (Table 4), suggesting that AGH harbors disulfide bond(s).

### Estimation of MW of AGH

MW of AGH was estimated by comparing the elution position of AGH with those of MW marker proteins by molecular sieve HPLC on a Superdex 75 HR 10/30 column. The AGH activity was eluted between 25 and 26 min and thus its molecular weight was estimated to be 11,000 ~ 13,000 (Fig. 5).

## DISCUSSION

In the previous experiment, we used male whole reproductive organs as starting material for the purification of AGH, and finally obtained two consecutive fractions by reverse-phase HPLC, referred to as AGH I and II (Hasegawa *et al.*, 1987). Subsequent studies revealed that AGH I contained mainly four structurally-related peptides produced specifically by the seminal vesicles and the vas deferens (Nagasawa *et al.*, 1995), indicating that the AGH I fraction was still impure. Since it was difficult to separate these peptides from AGH, we

**Table 2.** Effect of heat treatment on AGH activity

AGH fraction			
Treatment	Amount (animal eq.)		
	0.06	0.3	1.5
Control	1/6	4/6	5/6
Acidic condition	2/8	3/7	4/6
Neutral condition	1/8	6/8	4/6
AG-testis extract			
Treatment	Amount (animal eq.)		
	0.1	0.3	1.0
Control	4/7	6/7	5/6
Acidic condition	2/7	4/6	3/6
Neutral condition	0/7	1/10	1/10

AGH fraction and AG-testis extract were heated at 100°C for 3 min under acidic or neutral condition. AGH fraction was obtained by a single HPLC of AG extract. AG-testis extract was prepared by homogenization in Ringer solution at pH 7.0. For acidic conditions these were adjusted to pH 2.0 by the addition of 10% TFA before heating. The controls were not heated. X/Y: X is the number of masculinized females, and Y is the total number of females alive after molting.

**Table 3.** Effect of trypsin digestion on AGH activity

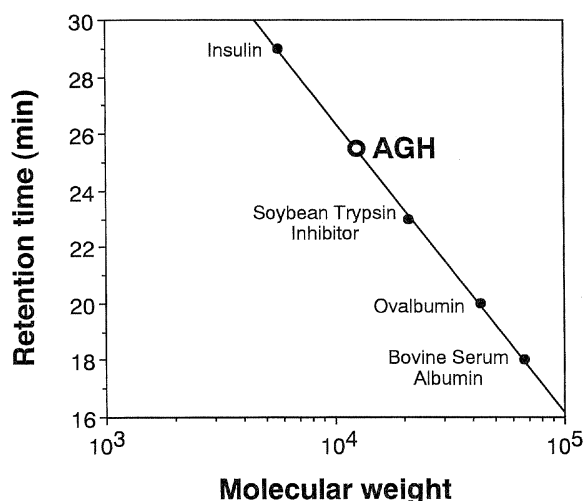
Treatment	Trypsin	Trypsin inhibitor	AGH fraction	Amount (animal eq.)	
				1.425	2.85
1	+	+	+	0/7	4/7
2	+	+	+	—**	0/9
3	—	+	+	2/10	5/9
4	—	—	+	4/9	3/5
5	+	+	—	0/4	

Each mixture was incubated at 37°C for 3 hr. +\* indicates that trypsin inhibitor was added after incubation. X/Y: X is the number of masculinized females, and Y is the total number of females alive after molting. —\*\*: not tested.

**Table 4.** Effect of reductive carboxymethylation to AGH activity

Treatment	Amount (animal eq.)			
	0.67	2.0	3.0	6.0
1	—	—	0/5	0/10
2	2/3	6/7	—	7/9
3	3/4	8/8	—	—

Treatment 1: The AGH fraction was incubated with dithiothreitol solution at 35°C for 30 min. Subsequently, sodium iodoacetate solution was added to the reaction mixture, and the incubation was continued at 35°C for 30 min. Treatment 2: The AGH fraction was incubated without reagents under the same conditions as in treatment 1. Treatment 3: untreated. X/Y: X is the number of masculinized females, and Y is the total number of females alive after molting. —: Not tested.



**Fig. 5.** Estimation of the MW of AGH. The MW of the AGH was estimated by molecular sieve HPLC on a Superdex 75 HR 10/30 column. Marker proteins used were insulin (MW, 5,700), soybean trypsin inhibitor (MW, 21,000), ovalbumin (MW, 43,000) and bovine serum albumin (MW, 67,000). The elution was performed with 0.2 M  $\text{NH}_4\text{HCO}_3$  at a flow rate of 0.5 ml/min at room temperature.

decided to shift the extraction material from using whole reproductive organs to using AGs only. This increased difficulties in carrying out the collection of AGs, and thus the efficiency of obtaining starting materials was lowered considerably. However, due to this shift, many impurities derived from the other parts of reproductive organs could be removed physically, which made the purification process much simpler. Thus, we could obtain a highly purified fraction of AGH via only three steps of HPLC.

In the present experiment, AGH activity was recovered in a single fraction at each step of purification by RP-HPLC. Thus, we think currently that there is only one molecular species of AGH. These results seem to be different from our previous results, in which two AGH fractions designated as AGH I and II were obtained by RP-HPLC. However, it is questionable that there were really two active principles in these fractions, because these two fractions were adjacent to each other and the UV peaks corresponding to AGH I and II fractions on the chromatogram did not represent AGH considering the low specific activity of these fractions as described later. Therefore, our present results are not necessarily inconsistent with the previous ones. The specific activity of the final preparation was 38 pg/unit, the value of which was about 1440- and 1870-times smaller than those of our previous preparations of AGH I and II, respectively. As far as we know, our final preparation in the present experiment had by far the highest specific activity among preparations thus far purified.

Instability of AGH in AG-testis extract to heat treatment under neutral conditions was consistent with the results by Katakura *et al.* (1975), who used the whole reproductive organs as experimental materials. By contrast, AGH fraction purified by RP-HPLC was tolerable to heating at 100°C for 3 min under neutral conditions. Besides, both preparations were

not affected by heat treatment under acidic conditions. These results might be explained by the existence of a factor in the testis capable of binding to AGH under neutral conditions but not under acidic conditions. And when heated, the complex might be precipitated only under neutral conditions. This idea could solve the discrepancy in terms of heat stability between the results obtained by Katakura *et al.* (1975) and Juchault *et al.* (1984), who used an AGH preparation from only AGs. Thus, we conclude that AGH is essentially heat-stable.

Concerning the chemical nature of AGH, proteinaceous property was represented by the fact that AGH was inactivated by proteolytic enzyme digestion (Katakura *et al.*, 1975). However, the enzymes after incubation were not removed nor inactivated before injection, and therefore it can not be ruled out that the enzymes themselves inhibited the masculinization. In the present experiment, to rule out the possibility that AGH was not digested by trypsin but instead that trypsin inhibited the masculinization, we used a trypsin inhibitor. Our results showed that trypsin did not affect AGH activity only in the presence of a trypsin inhibitor, supporting the previous results that AGH is a protein.

Reductive carboxymethylation caused almost complete loss of AGH activity, indicating the presence of disulfide bridge(s) in the AGH molecule. This is inconsistent with the results obtained by the AGH from hypertrophied AGs of intersexed animals, in which it retained activity after treatment with 5%  $\beta$ -mercaptoethanol in SDS-PAGE and cystine was not detected by amino acid analyses (Martin *et al.*, 1990). However, considering that cystine is usually recovered in a very poor yield by acid hydrolysis, the AGH could contain cystine residue(s). If so, it is also possible to assume that AGH once reduced was air-oxidized to the native form by refolding. But complete recovery of activity was unlikely.

The molecular weight of AGH estimated in the present study is different from those estimated previously. Estimation of MW seems to depend on the methods. Katakura *et al.* (1975) used gel-filtration on Sephadex G-75 (MW, 15,000 ~ 17,000), Juchault *et al.* (1978, 1984) used dialysis (MW, 2,000 ~ 8,000), and Martin *et al.* (1990) used SDS-PAGE (MW, 17,000 ~ 18,000). In the present experiment, we used molecular sieve HPLC, which had a higher resolution than open column gel-filtration. In fact, a good linearity between elution volume and logarithm of molecular weight of markers was observed, and AGH was eluted at positions corresponding to 11,000 ~ 13,000. In order to determine the exact value for MW of AGH, we have to wait until the complete structure will be established.

## ACKNOWLEDGMENTS

We are grateful to Prof. Y. Katakura for valuable advice on the sampling of androgenic glands. We also thank Dr. M. N. Wilder of Japan International Research Center for Agricultural Sciences for critical reading of this manuscript. This work was supported partly by a Grant-in-Aid for Scientific Research (No. 09460055) from the Ministry of Education, Science, Sports and Culture of Japan.

## REFERENCES

- Berreur-Bonnenfant J, Meusy JJ, Féréxou JP, Devys M, Quesneau-Thierry A, Barbier M (1973) Recherches sur la sécrétion de la glande androgène des Crustacés Malacostracés: Purification d'une substance à activité androgène. *CR Acad Sci Ser D* 277: 971–974
- Chaigneau J, Juchault P (1979) Quelques aspects ultrastructuraux de glandes androgènes hypertrophiées d' *Armadillidium vulgare* Latreille (Crustacé Isopode Oniscoïde), dans différents états de fonctionnement naturels ou induits. *Bull Sic Sci Nat Tunisie* 14: 29–34
- Charniaux-Cotton H (1972) Recherches récentes sur la différenciation sexuelle et l'activité génitale chez divers Crustacés supérieurs. In "Hormones et Différenciation Sexuelle Chez les Invertébrés" Ed by E Wolff, Gordon and Breach, Paris/London/New York, pp 127–178
- Charniaux-Cotton H, Payen G (1985) Sexual differentiation. In "The Biology of Crustacea Vol 9" Ed by DE Bliss and LH Mantel, Academic Press, New York, pp 217–299
- Férézou JP, Berreur-Bonnenfant J, Meusy JJ, Barbier M, Suchy M, Wipf HK (1977) 6,10,14-Trimethylpentadecan-2-one and 6,10,14-trimethyl-5-*trans*,9-*trans*,13-pentadecatrien-2-one from the androgenic glands of the male crab *Carcinus maenas*. *Experientia* 33: 290
- Hasegawa Y, Haino-Fukushima K, Katakura Y (1987) Isolation and properties of androgenic gland hormone from the terrestrial isopod, *Armadillidium vulgare*. *Gen Comp Endocrinol* 67: 101–110
- Hasegawa Y, Hirose E, Katakura Y (1993) Hormonal control of sexual differentiation and reproduction in Crustacea. *Amer Zool* 33: 403–411
- Juchault P, Maissiat J, Legrand JJ (1978) Caractérisation chimique d'une substance ayant les effets biologiques de l'hormone androgène chez le Crustacé isopode terrestre *Armadillidium vulgare* Latreille. *CR Acad Sci Ser D* 286: 73–76
- Juchault P, Legrand JJ, Maissiat J (1984) Present state of knowledge on the chemical nature of the androgenic hormone in higher crustaceans. In "Biosynthesis, Metabolism and Mode of Action in Invertebrate Hormones" Ed by J Hoffman and M Porchet, Springer-Verlag, Berlin/Heidelberg, pp 155–160
- Katakura Y (1984) Sex differentiation and androgenic gland hormone in the terrestrial isopod *Armadillidium vulgare*. In "The Biology of Terrestrial Isopods" Ed by SL Sutton and DM Holdich, Oxford Univ Press, Oxford, pp 127–142
- Katakura Y, Fujimaki Y, Unno K (1975) Partial purification and characterization of androgenic gland hormone from the isopod crustacean, *Armadillidium vulgare*. *Annot Zool Japon* 48: 203–209
- Katakura Y, Hasegawa Y (1983) Masculinization of females of the isopod crustacean, *Armadillidium vulgare*, following injections of an active extract of the androgenic gland. *Gen Comp Endocrinol* 48: 57–62
- King DS (1964) Fine structure of androgenic gland of the crab, *Pachygrapsus crassipes*. *Gen Comp Endocrinol* 4: 533–544
- Malo N, Juchault P (1970) Contribution à l'étude des variations ultrastructurales de la glande androgène des Oniscoïdes supérieurs (Crustacés Isopodes), à la suite de la décérébration. *CR Acad Sci Ser D* 271: 230–232
- Martin G, Juchault P, Sorokine O, Van Dorselaer A (1990) Purification and characterization of androgenic hormone from the terrestrial isopod, *Armadillidium vulgare*. *Gen Comp Endocrinol* 80: 349–354
- Meusy JJ (1965) Contribution de la microscopie électronique à l'étude de la physiologie des glandes androgènes d'*Orchestia gammarella* P. (Crustacé Amphipode) et de *Carcinus maenas* L. (Crustacé Décapode). *Zool Jb Physiol Bd* 71: 608–623
- Nagasawa H, Hasegawa Y, Haino-Fukushima K, Hatayama H, Yanagisawa T, Katakura Y (1995) Isolation and structural determination of seminal vesicle-specific peptides of the terrestrial isopod, *Armadillidium vulgare*. *Biosci Biotech Biochem* 59: 1246–1250
- Sutton, SL (1972) Investigations. In "Woodlice" Ed by SL Sutton, Pergamon Press, Oxford/New York/Toronto/Sydney/Paris/Frankfurt, p 115

(Received April 18, 1997 / Accepted June 23, 1997)