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METHYL FARNESOATE IN THE PRAWN *MACROBRACHIUM ROSENBERGII*: SYNTHESIS BY THE MANDIBULAR ORGAN *IN VITRO*, AND TITERS IN THE HEMOLYMPH

AMIR SAGI, ELLEN HOMOLA and HANS LAUFER

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06268, USA (Fax: 203 486 4331); and The Marine Biological Laboratory, Woods Hole, MA 02543, USA

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Abstract—1. The mandibular organ of *Macrobrachium rosenbergii* was identified and cultured in the presence of [methyl-^{3}H]-methionine.

2. Radiolabelled methyl farnesoate was extracted and quantitated from cultured glands and culture media of both males and females: variation in synthesis rates was observed (1558–52,652 DPM/hr per gland) between individual prawns.

3. No significant incorporation of isotope into hexane-extractable material was observed in any other tissue cultured.

4. The concentration of methyl farnesoate in the hemolymph of *M. rosenbergii* was determined using normal phase HPLC with *cis–trans* (non-biological isomer) as an internal standard.

5. Hemolymph from males contained 17.3 ± 9.5 ng/ml methyl farnesoate.

6. Female hemolymph contained 24 ± 13.1 ng/ml methyl farnesoate.

INTRODUCTION

The mandibular organ was first described by Le Roux (1968). Two decades later, Laufer *et al.* (1987) identified a secretory product of this gland, methyl farnesoate. Since then, methyl farnesoate has been found in many crustaceans including crabs (Laufer *et al.*, 1987), lobsters (Laufer and Borst, 1988) and crayfish (Landau *et al.*, 1989). Methyl farnesoate is the unepoxidated form of the insect juvenile hormone, JH III. In insects, juvenile hormones play several regulatory roles both as gonadotropins (Herman and Bennett, 1975) and morphogens (Wigglesworth, 1970).

Macrobrachium rosenbergii is a decapod crustacean with a unique social structure (Ra'anan and Cohen, 1985), characterized by the coexistence of distinct reproductive and morphological types (Ra'anan and Sagi, 1985). Thus, this organism could serve as an interesting model for the study of methyl farnesoate function in the regulation of reproduction and morphogenesis in crustacea. The present study represents the first report of the identification and quantification of methyl farnesoate in the hemolymph and the localization of its site of synthesis and secretion, the mandibular organ, in the freshwater prawn *M. rosenbergii*.

MATERIALS AND METHODS

Animals

Male and female *M. rosenbergii*, 20–35 g body weight, were obtained from Aquaculture Enterprises in Sabana Grande, Puerto Rico. The prawns were acclimated for at least 1 week before experiments were undertaken. Acclimation was done in a 500 gallon freshwater tank. The water was recirculated through a gravel biofilter and the temperature kept between 25°C and 28°C. The prawns were fed daily frozen squid and commercial prawn pellets (P.D.M. and Associates, Norwich, CT). Under these conditions the social behaviour of males and ovarian maturation in females, seen in pond grown populations, were observed in the laboratory.

Mandibular organ culture in vitro

Mandibular organs were dissected and cultured attached to the mandibular tendon. Similarly sized fragments of muscle, connective tissue and androgenic glands were cultured as controls. Each tissue was cultured in 400 μl of media for 2 hr at 30°C with slight agitation. Culture medium was *M. rosenbergii* saline (Nagamine *et al.*, 1980) containing 20 mM HEPES (pH 7.6), 3.8 mM dextrose and 0.2% BSA. [Methyl-^{3}H]-methionine (ICN) served as a radiolabelled methyl group donor for methyl farnesoate synthesis. The spec. act. was 200 mCi/mmole, and total activity in the culture media was 40 μCi/ml (Laufer *et al.*, 1987). Following incubation, the tissues were fixed with 0.5 ml ethanol. The tissues were homogenized in ethanol, pooled with the incubation media, added to a 6 ml volume of 4% NaCl and extracted with 1 ml hexane. One hundred μl of the hexane extract and 30 ng of cold all-*trans* methyl farnesoate standard were loaded onto a Waters HPLC, with model 501 pumps, equipped with a 5u Econosil silica column (Alltech). The running solvent was 1% diethyl ether in hexane flowing at 2.5 ml/min. The absorbance detector (Lambda Max 481) was set at 218 nm and fractions were collected every 30 sec. The activity in a 100 μl aliquot of the hexane extract and the HPLC fractions were counted in Scintilene (Fisher) using a Backman liquid scintillation spectrometer. This method was used to determine the percentage of radioactivity which co-eluted with the methyl farnesoate standard. Subsequently, a modified procedure, developed by Feyereisen and Tobe (1981) for juvenile hormone synthesis was used, in which separation by phase partitioning is followed by liquid scintillation and separation by HPLC is eliminated since no other radioactive peaks were observed in the hexane phase.

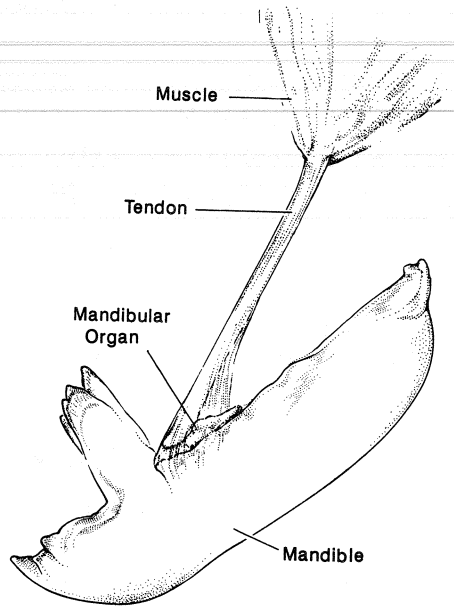

Fig. 1. The location of the mandibular organ in *M. rosenbergii*. The figure was drawn from a dissected large (90 g) male.

Methyl farnesoate in the hemolymph

Hemolymph samples (2 ml) were taken from the cephalothorax using a 3 cc syringe and 18 ga needle prerinsed with 0.01% EDTA. The samples were transferred to 15 ml Kimax culture tubes, on ice, containing 5 ml acetontrile and 2 ml 4% NaCl. Twenty-five ng of the *cis–trans* (non-biological) isomer of methyl farnesoate was added to each tube as an internal standard. The samples were extracted twice with 0.5 ml hexane. Ten μl of the hexane fraction was loaded onto a Hewlett Packard gas chromatograph with a bonded dimethyl silica column (100–250°C, 5.0°C/min) equipped with a mass spectrometer. Hemolymph titers of methyl farnesoate were determined according to the method developed by Laufer *et al.* (1987) and modified by Tsukimura *et al.* (1989). Two hundred μl from the hexane phase were loaded on to a Waters HPLC (conditions as described above). Peak areas were calculated using omega software (Version 1.4 Perkin Elmer/Nelson) and hemolymph methyl farnesoate titers were determined with reference to the known amount of internal standard in the sample.

RESULTS

The mandibular organ of *M. rosenbergii* was identified as a small structure (Fig. 1) associated with the junction of the mandibular tendon and the mandible. *In vitro* culture of this structure, in the presence of [methyl ^{3}H]methionine, resulted in the synthesis of a radiolabelled product which on HPLC and GC–MS analysis proved to be predominantly methyl farnesoate. Table 1 represents the amounts of radiolabeled material produced by a mandibular organ within a 2 hr incubation period. Separation of the synthetic products of the mandibular organ using normal phase HPLC revealed that the radioactivity co-eluted with methyl farnesoate (see Fig. 2 dark bar). The methyl farnesoate peak represents 87.7% of the total amount of radioactivity injected. Other tissues, both muscle and connective, associated with the mandibular tendon, as well as androgenic gland tissue were cultured and failed to show methyl farnesoate synthetic activity using the same radiolabelling procedure.

Table 1. Radiolabeled product synthesized *in vitro* by *M. rosenbergii* tissues in the presence of [methyl^{3}H]methionine

Tissue cultured source	*n*	Radioactivity in product (DPM/hr/gland or tissue) Min	Max	Average
Male MO	12	1558	52652	13,908 ± 18,458
Female MO	7	1720	38696	12,699 ± 13,339
Muscle	5	241	456	370 ± 47
Tendon	5	153	463	261 ± 69
Androgenic gland	4	285	772	447 ± 229

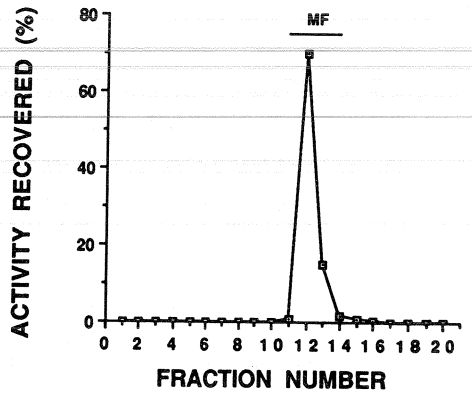

Fig. 2. The secretion of radiolabeled methyl farnesoate by *M. rosenbergii* mandibular organ *in vitro*. The figure shows the analysis of products synthesized by a mandibular organ from a male with a total count of 150,565 dpm/4 hr/gland.

Males and females produced similar amounts of methyl farnesoate. The amounts produced by individual prawns varied widely in both sexes. Assuming that the spec. act. of the radiolabelled methyl farnesoate synthesized by the gland is equal to that of the precursor and that only one labeled methyl group is incorporated into each methyl farnesoate molecule, the *in vitro* production rate of the mandibular organs of *M. rosenbergii* was calculated (Table 3) as was previously calculated for *Libinia emarginata* (Laufer *et al.*, 1987) and *Procambarus clarkii* glands (Landau *et al.*, 1989). The hexane fraction of the hemolymph, which contained known amounts of the non-biological, *cis–trans* methyl farnesoate isomer (internal standard, IS, Fig. 3), was separated using the HPLC. The methyl farnesoate peak (MF, Fig. 3) was identified by comparing its retention time with an external standard (all-*trans*) methyl farnesoate on HPLC. Gas chromatography and mass spectrometry, of the putative peak, showed a typical methyl farnesoate profile. The amounts of methyl farnesoate in the hemolymph were calculated using relative peak areas and are presented in Table 2. Females and males have almost the same average methyl farne-

Table 2. Concentrations of methyl farnesoate in *M. rosenbergii* hemolymph

Source	*n*	Hemolymph methyl farnesoate concentration (ng/ml) Min	Max	Average ± SD
Males	20	0	37.2	17.3 ± 9.5
Females	6	0	40.0	24.0 ± 13.1

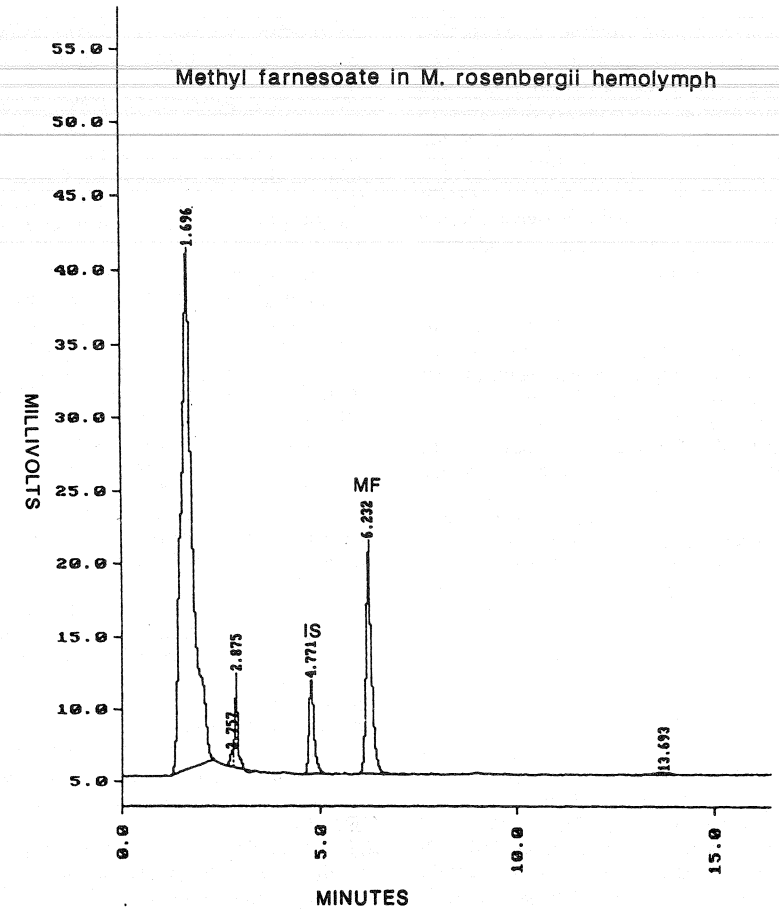

Fig. 3. Analysis of *M. rosenbergii* hemolymph for methyl farnesoate. The figure shows the analysis of hemolymph from a premolt male using *cis–trans* methyl farnesoate as an internal standard (IS). The calculated methyl farnesoate titer for this specimen was 32.6 ng/ml.

soate titer and in both sexes the heterogeneity among individual animals (0–40 ng/ml) was wide.

DISCUSSION

This is the first report to our knowledge on mandibular organ and its synthetic product, methyl farnesoate, in the freshwater prawn *Macrobrachium rosenbergii*.

Production of methyl farnesoate by cultured glands from males and females produced amounts of methyl farnesoate which were not significantly different between the sexes. The variability among individuals was very wide (3.5–119.6 pmoles per hr per gland). These synthesis rates are shown in Table 3 and are compared with other species in which *in vitro* synthesis has been calculated. Synthetic rates in *M. rosenbergii* were lower than the rates reported for *L. emarginata* (Homola, 1989), slightly higher than those reported for crayfish (Landau *et al.*, 1989) and several other crustacean species (Laufer and Borst, 1988). However the latter researchers were measuring only the amounts secreted into the incubation media. Methyl farnesoate secretion by *L. emarginata* was estimated to be 10% of the total synthetic methyl farnesoate produced, in a 2 hr period, by a mandibular organ *in vitro* (Homola, 1989).

Methyl farnesoate titers in the hemolymph varied widely between individual *M. rosenbergii*. Unlike *L. emarginata* (Laufer *et al.*, 1987), titers were higher in females than in males; however the differences were not statistically significant. The wide differences that were found within each of the sexes in both synthesis rates and hemolymph titers of methyl farnesoate

Table 3. Calculated *in vitro* synthesis rates of *M. rosenbergii* mandibular organ and a comparison of the rates among eight species of crustaceans

	Methyl farnesoate synthesis rates (pmol/hr/gland)			
Tissue source	*n*	Min	Max	Average ± SD
M. rosenbergii				
males	12	3.5	119.6	31.6 ± 41.9
females	7	3.9	87.9	28.9 ± 30.3
*L. emarginata**				
juvenile female	98			125 ± 56
adult female	8			125 ± 35
adult males	18			771 ± 500
P. clarkii†				14.6
L. emarginata‡		1.2	16.0	
C. meanas‡		0.7	23.9	
C. boralis‡		3.1	9.3	
C. bartonii‡		0.5	2.0	
H. americanus‡		0	0.13	

*Methyl farnesoate synthesis rates (Homola, 1989 and Homola, unpublished data).
†Methyl farnesoate synthesis calculated for untreated glands from Landau *et al.* (1989).
‡Methyl farnesoate secretion (Laufer and Borst, 1988).

could reflect differences in reproductive states, as was shown for female spider crabs (Laufer *et al.*, 1987). These varying levels may reflect different functional and morphological states as was recently described in male spider crabs (Laufer *et al.*, 1990; Sagi *et al.*, 1990). Since distinguishable reproductive and morphological types coexist in *M. rosenbergii* populations, the question of a relationship between methyl farnesoate titers, morphogenesis and reproductive state will be addressed in future studies.

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