L-Ovothiol A: The Egg Release Pheromone of the Marine Polychaete *Platynereis Dumerilii*: Annelida: Polychaeta

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Sex pheromones, released with the coelomic fluid by male *Platynereis dumerilii* initiate egg release in swarming females. The egg release pheromone, isolated from the coelomic fluid of sexually mature males, was identified as L-Ovothiol A, which was found in male marine invertebrates for the first time. Isolation was obtained by reversed-phase high-performance liquid chromatography in the biologically inactive disulfide form.

Introduction

Although there is a lot of experimental evidence for sex pheromones in marine invertebrates, only a few structures have been chemically elucidated (Kittredge *et al.,* 1971, Zeeck *et al.,* 1988, 1996, 1998a,b,c). The chemical nature of these substances is strongly differing. Uric acid as the sperm release pheromone (SRP) of *Platynereis dumerilii* (Zeeck *et al.,* 1998a) and inosine as the main component of the egg release pheromone (ERP) of *Nereis succinea* (Zeeck *et al.,* 1998b) belong to the family of purine ring systems. The SRP of *Nereis succinea* on the other hand derives from glutathione and is L-cysteine-glutathione disulfide (Zeeck *et al.,* 1998c).

In *Platynereis dumerilii*, the characteristic reproductive behaviour is synchronised by a consecutive release of male and female sex-specific pheromones. The source is the coelomic fluid of the opposite sex (Boilly-Marer, 1974, 1986). In the moment of detection of a swarming female, achieved by chemical signals, males discharge a small sperm cloud along with the ERP (Boilly-Marer, 1974). The ERP stimulates the female to swim fast in narrow circles around the sperm cloud, and after a short period (up to 10 seconds) the female begins to spawn. The emitted egg cloud contains the SRP. Fertilisation is achieved by attracted males circling around the eggs and discharging great amounts of sperm.

Here we report the isolation and identification of the ERP of *Platynereis dumerilii*, present in the coelomic fluid of sexually mature males.

Experimental

Sexually mature females and males of *Platynereis dumerilii* for pheromone identification as well as for behavioural bioassays were obtained from our laboratory culture according to the method of Hauenschild and Fischer (1969). Atoke species were collected from the Basin of Arcachon and the culture was refreshed yearly. Natural seawater (salinity 3.2%) was obtained from the North Sea near Helgoland, filtered over charcoal and pasteurized by heating to 80 °C for 30 minutes.

Coelomic fluid of males was obtained by carefully pressing along the worms with tweezers. Isolation of the ERP was achieved by ultrafiltration (Amicon Diaflo membranes) of coelomic fluid samples and bioassay-guided fractionation of the aqueous filtrate with reversed-phase high-performance liquid chromatography equipped with a semipreparative column (LiChrospher 100 RP18e, 10 µmm, 250×10 mm, Merck), using a gradient water/(methanol/water 60/40, v/v), flow 4ml/min. The chromatographic purity of the reduced disulfide was checked with water isochratic on an analytical column (LiChrospher 100 RP18e, 5 µmm, 250×4 mm, Merck), flow 0.6ml/min.

The biologically active fraction was eluted between 3.5 to 4.2 minutes, but the activity decreased to zero whilst removing the water. The now biologically inactive fraction was rechromatographed on the same chromatographic system and a new compound was eluted after 11.5 minutes (Fig. 1) and was assumed as a degradation product of the active sample.

Qualitative bioassays were carried out at a temperature of 18–20 °C. Samples were injected with a 10 µml microlitre syringe (Hamilton) in front of a mature female swarming in a 60ml glass dish filled with 40ml pasteurised sea water. The sponta-

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neous release of the coelomic fluid along with the eggs represented a positive behavioural response.

Results and Discussion

L-Ovothiol A disulfide was identified as the degradation product of the ERP of *Platynereis dumerilii*. The structure was deduced by comparison of its ^{1}H-, ^{13}C-, HMBC- HMQC-NMR (Table I), ESI–MS- and UV/VIS-spectra, with data published in literature (Holler *et al.*, 1989; Palumbo *et al.*, 1982; Burgoyne *et al.*, 1991). It was expected that the biologically active substance is represented by L-ovothiol A (L-1-methyl-4-mercaptohistidin) and that the disulfide is formed by oxidation of L-ovothiol A.

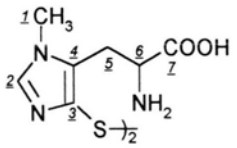

Table I. ^{1}H and ^{13}C data of ovothiol A disulfide recorded in D_2O at ambient temperature with 500 MHz.

^{13}C ^{1}H Position	^{1}H	^{13}C
1	3.56 s	32.7
2	7.78 s	141.0
3	–	131.0
4	–	133.8
5	2.68 dd (7.5) 2.82 dd (7.5)	24.8
6	3.60 t (7.5)	54.1
7	–	173.0

After treating the disulfide with sodiumborhydride (Palumbo *et al.*, 1982) the highly active reduced form of the amino acid was recovered. The free amino acid and the active fraction of fresh male coelomic fluid showed equal characteristics on several chromatographic systems, identical behavioural response in bioassays and similar UV/VIS-spectra (Fig. 1).

Recently L-ovothiol A was discovered in eggs only, never in male coelomic fluid, of marine invertebrates (Palumbo *et al.*, 1982). Originally the structure was incorrectly identified postulating the methyl substituent on N-3 position of the imidazole ring. It was later corrected by Holler *et al.* (1987). Free L-ovothiol A is proposed to protect sea urchin eggs from the oxidative stress, caused by the respiratory burst which takes place immediately after fertilisation. Sea urchin eggs produce extracellular H_2O_2 which is used in the construction of an envelope to protect the early embryo by cross-linking tyrosyl residues (Shapiro and Turner, 1988). Consuming the H_2O_2 that crosses the plasma membran leads to the formation of the L-ovothiol A disulfide, that is reduced by glutathione. So, L-ovothiol A can act like a glutathione – peroxidase and replaces the function of this enzyme (Turner *et al.*, 1988, Saphiro 1991).

But this amino acid seems to have no function for sperm cells and, therefore, can accumulate in the coelomic fluid of males. This accumulation is a precondition for using L-ovothiol A as a pheromone. Thiessen pointed out that a minimised energetical effort is the great advantage of using an existing biochemical pathway to form a pheromone (Thiessen, 1977).

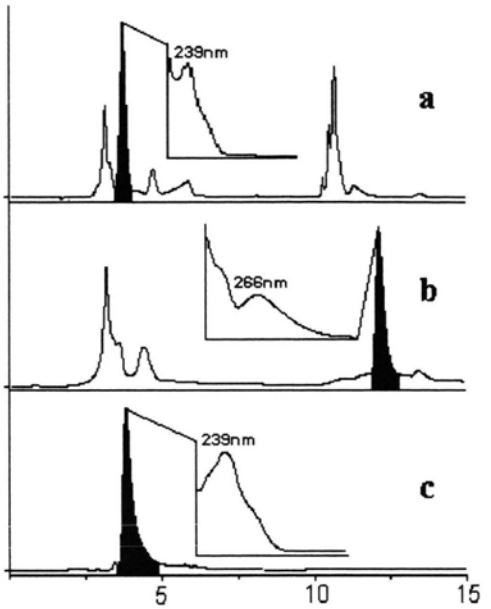

Figure 1

Fig. 1. **a**) Chromatogram of male coelomic fluid. Black area corresponds to the active fraction. **b)** Chromatogram of the oxidized active fraction from **a** containing the disulfide (black area). **c)** Chromatogram of the disulfide fraction from **b** after reduction with $NaBH_4$. In addition UV spectra are indicated.

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