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Ovarian jelly-peptides (OJPs), a new family of regulatory peptides identified in the cephalopod *Sepia officinalis*

Benoît Bernay [a], *Michèle Baudy-Floc'h* [b], *Jean Gagnon* [c], *Joël Henry* [a,*]

[a] *Laboratoire de Biologie et Biotechnologies Marines, UMR 100 IFREMER Physiologie et Ecophysiologie des Mollusques Marins, Université de Caen, 14032 Caen Cedex, France*

[b] *Laboratoire SESO, UMR CNRS 6510, Institut de Chimie, Université de Rennes I, 263 Av. du Général Leclerc, F-35042 Rennes Cedex, France*

[c] *Laboratoire d'Enzymologie Moléculaire, Institut de Biologie Structurale, Jean-Pierre Ebel, CEA-CNRS-UJF, 41 rue Jules Horowitz, 38027 Grenoble, France*

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ABSTRACT

In marine invertebrates, numerous water-borne peptides involved in reproductive behavior have been characterized. In this study, we focused on three ovarian water-borne peptides, released by full-grown oocytes (FGO) in the genital coelom and in the lumen of the oviduct in the cuttlefish *Sepia officinalis*. The first one (DQVKIVL), was characterized by the monitoring of HPLC purified fraction using a myotropic bioassay. Subsequently, a peptidomic approach consisting of a mass spectrometry comparative screening performed between the peptide content of FGO with that of FGO-conditioned medium, led to the identification of two additional water-borne peptides. The second peptide identified (DEVKIVL) was characterized by MS/MS and the primary structure of the third one (DEVKIVLD) was elucidated by a combination of Edman degradation, acid hydrolysis and MS/MS analysis. Sequence homology, tissue mapping and bioactivity demonstrate that these peptides belong to the same family. DQVKIVL-related-peptides strictly localized in the female genital tract modulate the whole female genital tract and the main nidamental gland contractions. Furthermore, these peptides form a jelly, when resuspended in water. This particular property could play an important role in the kinetics of peptide diffusion in the external medium. Thus, these regulatory peptides were named ovarian jelly-peptides (OJPs).

1. Introduction

Since the introduction of the term "pheromone" by Karlson and Luscher [15], a number of water-borne peptides and proteins which play an important role in the coordination of the reproductive behavior have been discovered in aquatic organisms. In protozoans, peptide pheromones are responsible for mating interactions [17]. In amphibians, sodefrin and silefrin, two sex-pheromone peptides characterized in *Cynops pyrrhogaster* and *C. ensicauda*, respectively [16,28] attracted conspecific females. In the polychaete worm *Nereis*, the peptide nereithione which modulates male nuptial behavior and induces sperm release, was isolated from the coelomic fluid of sexually mature females [21,35]. In cnidaria and echinodermata, more than 80 sperm attracting and activating peptides (SAPs), are released by

* *Corresponding author*. Tel.: +33 2 31 56 55 96; fax: +33 2 31 56 43 56.
E-mail address: joel.henry@unicaen.fr (J. Henry).

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the eggs in seawater to facilitate fertilization by increasing chances of gamete collision [10,18,23,26]. In molluscs, water-borne peptides and proteins have also been identified from gastropods and cephalopods. In *Aplysia*, at least four water-borne peptides, attractin, temptin, enticin and seductin, are released by the egg cordons to induce aggregation of mature adults and egg-laying [6,7,19,20]. In the cuttlefish *Sepia officinalis*, numerous water-borne peptides and one neuromediator able to modulate the successive steps of egg-laying, are released by oocytes in the genital coelom as well as in the lumen of the oviduct [31–33]. The peptidomic or peptide display technique which consists of the comparison between the peptide content of different samples, is a powerful means of identification of putative regulatory peptides. In the cuttlefish *S. officinalis*, this experimental strategy led to the characterization of *Sepia* capsule releasing peptide (SepCRP), a water-borne peptide released by the full-grown oocytes (FGO) in the genital coelom, and involved in the successive steps of egg-laying [2]. After the release of FGO in the genital coelom (ovulation) [9], the ovarian 5-HT and the SepCRP released by the FGO inhibit the contractions of the oviduct until mating [2,31]. After mating, the oocytes are transported by the oviduct contractions controlled by neuropeptides [11,12] and ovarian peptides [32,33]. Then, FGO receive a first gelatinous envelope secreted by the oviduct gland and a second one secreted by the nidamental glands [14]. Finally, oocytes are fertilized by spermatozoa stored in the copulatory pouch and fertilization is facilitated by ovarian peptides [3,34]. In this context, we focused on water-borne peptides expressed in the ovary and targeting oviduct and accessory sex glands. By means of mass spectrometry screening and HPLC purification, we identified three novel ovarian peptides putatively involved in the regulation of egg-laying. Primary sequence, tissue mapping, activity and chemical properties demonstrate that these peptides belong to a new single family.

2. Materials and methods

2.1. Animals

All the cuttlefish were trapped in the Bay of Seine between January and June. They were maintained in 1000 L outflow tanks at 15 °C ± 1 °C at the Marine Station of Luc sur Mer (University of Caen, France) under a natural photoperiod.

2.2. Recovery of material from tissues and seawater

For HPLC purification and microLC-ESI-MS/MS analysis, 600 g of FGO were homogenized in 6 L of 0.1N HCl at 100 °C and centrifuged 30 min at 35,000 × *g* at 4 °C. The supernatants were concentrated on Chromafix C18 cartridges. For the microLC-ESI-MS/MS analysis, previtellogenic follicles, vitellogenic follicles and eggs were extracted as described above. Moreover, the molecules released from 50 FGO or 50 vitellogenic oocytes in 20 mL of synthetic filtered seawater were concentrated after various incubation times on Chromafix C18 cartridges to be analyzed in microLC-ESI-MS/MS.

2.3. MicroLC-ESI-MS/MS analysis

Analyses were performed with a HPLC Surveyor chain connected on-line to an orthogonal electrospray source (Deca XP MS-n Thermofinnigan) operated in the positive electrospray ionization mode (ESI+). The ions were focused into an ion trap, capable of MS and MS/MS analyses. The mass spectra were acquired during 35 ms from *m/z* 300 to 2000. The capillary exit of the electrospray ion source was set at 70 V, the octapole at 3 V and the capillary temperature at 200 °C. A counter flow of nitrogen was used as nebulizing gas. Xcalibur data system was used to acquire the data, which were further processed with the Turbo Sequest data system. The organic fraction of each extract was resuspended in 10 μL of 0.1% formic acid in water and injected onto a C18 Thermo Hypersil column (50 mm × 0.5 mm, 3 μm) with an acetonitrile (ACN) linear gradient of 3%/min in 0.1% formic acid, from 2 to 60%. A split ratio of 30:1 was used to perfuse the column at a flow rate of 10 μL/min. The HPLC column was rinsed with 90% ACN in 0.1% formic acid between each injection. The MS data was acquired in scan mode considering the positive ion signal.

2.4. LC-ESI-MS purification

The FGO extract was resuspended in 100 μL of 0.1% formic acid in water and injected into Nucleosil C18 column (250 mm × 4 mm, 7 μm) with an ACN linear gradient of 0.36%/min in 0.1% formic acid at a flow rate of 1 mL/min, during 25 min. A split ratio of 100:1 was used to perfuse the electrospray source at a flow rate of 10 μL/min and 1 min fractions were collected. The second step was performed in UV-HPLC. The bioactive fraction eluted at 22.20 min was injected onto a Nucleosil C18 column (250 mm × 4 mm, 5 μm) with an ACN linear gradient of 1.33%/min in 10 mM ammonium acetate at a flow rate of 1 mL/min.

2.5. UV-HPLC purification

HPLC analysis was performed with a Varian 4050 integrator connected to a Varian 9012 solvent delivery system and a Varian 9050 wavelength UV–vis detector set at 214 nm. FGO-conditioned seawater was resuspended in 0.1% formic acid in water and injected into a Nucleosil C18 column (250 mm × 3 mm, 7 μm) with an ACN linear gradient of 1.33%/min in 0.1% formic acid at a flow rate of 1 mL/min, during 45 min from 0 to 60% ACN. One minute HPLC fractions were dried and kept at 4 °C until use.

2.6. Edman degradation

N-terminal sequence analyses were performed using an Applied Biosystems Model 477 A protein sequencer, and amino acid phenylthiohydantoin derivatives were identified and quantified on-line with a Model 120 A HPLC system, as recommended by the manufacturer. The amino acid sequence was checked from MS/MS spectrum with the softwares