Peptidomics: a new omics-sibling in ecotoxicology?

Steven J. Husson[1,2], Kristien A. Van Camp[1], Geert Baggerman[1,2,3], Ronny Blust[1,2]

[1] Systemic Physiological and Ecotoxicological Research (SPHERE), Department of Biology, University of Antwerp, Groenenborgerlaan 171/U7, B-2020 Antwerpen, Belgium
[2] Centre for Proteomics (CFP), UAntwerp - VITO, Groenenborgerlaan 171, B-2020 Antwerp, Belgium
[3] Flemish Institute for Technological Research (VITO), Boeretang 200, B-2400 Mol, Belgium

E-mail contact: steven.husson@uantwerpen.be

1. Introduction

(Neuro)peptides are small messenger molecules that are derived from larger, inactive precursor proteins by the highly controlled action of processing enzymes. These biologically active peptides can be found in all metazoan species where they orchestrate a wide variety of physiological processes. Obviously, detailed knowledge on the actual peptide sequences, including the potential existence of truncated versions or presence of post-translation modifications, is of high importance when studying their function. A peptidomics approach therefore aims to identify and characterize the endogenously present peptide complement of a defined tissue or organism using liquid chromatography and mass spectrometry. While the zebrafish *Danio rerio* is considered as an important aquatic model for medical research, neuroscience, development and ecotoxicology, very little is known about their peptidergic signaling cascades. We therefore set out to biochemically characterize endogenously present (neuro)peptides from the zebrafish brain using a "peptidomics" workflow (Figure 1).

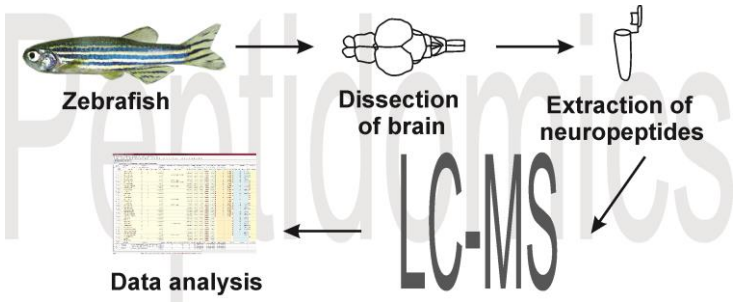

Figure 1: Peptidomics workflow

2. Materials and methods

The entire brain was dissected as quickly as possible and immediately placed in ice cold peptide extraction solvent consisting of methanol:water:acetic acid (90:9:1; v:v:v). The sample was kept on ice and sonicated using a bar sonicator prior to centrifugation. The supernatant was collected from which the methanol was evaporated by using a Speedvac concentrator. Lipids were removed from the remaining aqueous residue, containing the peptides, by re-extraction with ethyl acetate and n-hexane. The aqueous solution was subsequently desalted by solid phase extraction using a C_{18} Spin Column and reconstituted in 10 µL water containing 2 % ACN and 0.1 % FA for LC-MS analysis.

LC-MS analysis was performed on a Eksigent nanoLC-Ultra system connected to a Thermo Scientific LTQ-Orbitrap Velos mass spectrometer. Separation was conducted using a linear gradient from 2 % ACN in water, 0.1 % FA to 40 % ACN in water, 0.1 % FA in 45 minutes; the flow rate was set at 350 nl/min. The LTQ-Orbitrap Velos was set up in a data dependent MS/MS mode where a full scan spectrum (350–5000 m/z, resolution 60000) was followed by a maximum of ten collision-induced dissociation (CID) tandem mass spectra (100 to 2000 m/z). Proteome discoverer (1.3) software (Thermo Scientific) was used to perform database searching against the NCBI database nr_20130601 filtered for taxonomy *Dano rerio* using Mascot. Only medium and high confident peptides with a global FDR < 5 % and first ranked peptides were included in the results.

3. Results and discussion

In a first attempt to biochemically identify endogenously present peptides from brain tissues of the zebrafish *Danio rerio*, a peptidomics workflow was employed. The entire brain region of 6 male adult zebrafishes were carefully dissected and 6 independent (neuro)peptide extracts were made using an extraction protocol that is extremely efficient in avoiding the presence of protein degradation products. The 6 peptide samples were analyzed using a nanoLC instrument that is directly coupled with an LTQ-Orbitrap mass spectrometer to yield biochemical identifications of 62 peptides that belong to 34 different peptide precursor proteins. In addition, a remarkable amount of shortened forms (aminoterminally or carboxyterminally truncated) could be identified, yielding a total number of 105 peptide identifications. These peptide variants may result from further *in vivo* processing in the vesicles or might be the result of extracellular (*in vivo*) peptide processing by specific peptidases. Alternatively, they can also occur from in vitro degradation during sample processing, as has previously been noted.

Since in data dependent analysis peptides are selected in a semi-stochastic way, repeated analysis will increase the number of peptides identified. Six independent peptidomics extracts and LC-MS experiments were performed from which accumulative amounts of peptide identifications are plotted in figure 2. From this figure, it shows that further increasing the number of additional LC-MS runs would only marginally increase the total amount of identified peptides. In fact, this graph suggests that, within the technical limits in terms of sensitivity, most if not all peptides present were sampled. Of course, our results also indicate that the measured peptide profiles of individual fish differ. It is therefore difficult to perform robust differential peptidomics studies in an ecotoxicological setting that e.g. aim at comparing the peptide content of exposed fish vs. control fish.

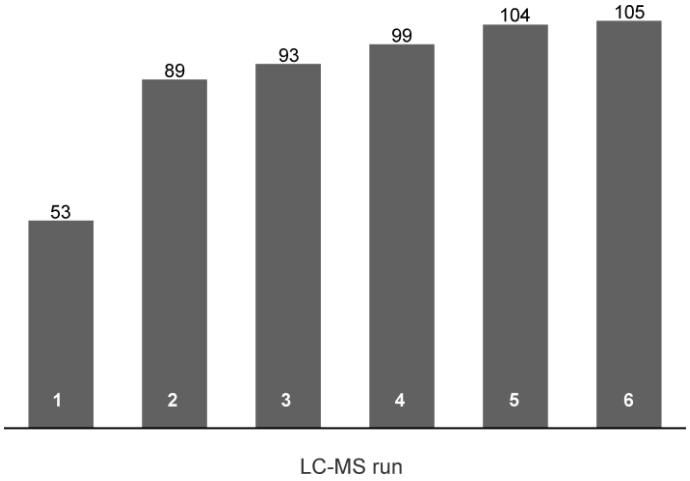

Figure 2: Accumulative count of unique peptide identifications from six LC-MS/MS analysis

4. Conclusions

The zebrafish is a well-established model organism to study vertebrate biology and gene functions. However, very little knowledge about the biochemical peptide entities was available. In the present study we analysed the endogenous peptides content from the zebrafish brain by LC-MS to identify 62 (neuro)peptides. To our knowledge, this is the first high-throughput peptidomics study of a fish species. As such, this archive of identified endogenous peptides is likely to aid future research in (neuro)endocrinology in this important model organism.

It is very important to realize that the endogenous peptide content of a cell, tissue or organism is spatially and temporally dynamic. Furthermore, remarkable inter-individual variations exist, even when using specific zebrafish strains. Taking these pitfalls together, application of in-depth differential peptidomics technologies in an ecotoxicological research setting e.g. to study modulatory effects of defined exposure scenarios on neuropeptidergic signalling systems is very challenging. However, this pioneering study can be considered as a basic blue print of endogenously present peptides in the fish brain, which is indispensable for further attempts to monitor changes in peptide expression in response to changes in the organism or the environment using differential peptidomics or molecular imaging techniques such as immunocytochemistry or MALDI imaging.

Acknowledgement - The authors highly appreciate funding from the University Research Fund (Bijzonder Onderzoeksfonds, BOF).