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Short communication

Mass spectrometric profiling – A diagnostic tool in fish?

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Abstract

The development of rapid and sensitive diagnostic tools to assess the effect of stressors on organisms is a principal objective of environmental proteomics. This study is focused on evaluating the potential of using surface-enhanced laser desorption/ionisation time-of-flight mass spectrometry (SELDI-TOF MS) to assess stress in Atlantic salmon (*Salmo salar*).

Plasma and mucus samples were taken from fish that had previously been maintained in a range of high density conditions, together with control fish maintained under low density conditions. Samples were collected during the post-density stress period for protein profile analysis. The mass spectra were analysed to evaluate reproducibility and to search for condition specific changes in protein expression. Multivariate analysis of the peak relative intensity data indicated a segregation of the data into three entities in accordance with the density level fish had been subjected to during the density stress period. This segregation was seen in both plasma and mucus data.

Keywords: Proteomics; Mucus; Atlantic salmon; SELDI-TOF MS

SELDI-TOF MS (Ciphergen Biosystems) is a high-throughput technique utilised to generate peptide/protein profiles and has been used in the medical field to identify disease specific biomarkers (Bischoff and Luider, 2004; Von Eggeling et al., 2001). Protein

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profiling using the SELDI-TOF MS instrument has also been utilised in environmental monitoring of marine species, to identify biomarkers of toxic exposure (Bjørnstad et al., 2006). The technology consists of a protein chip array, the laser desorption/ionisation time-of-flight mass spectrometer (LDI-TOF MS) and the software which facilitates analysis of the spectra. Due to the specific binding capacity of the protein chip array a subset of proteins from the sample is presented to the LDI-TOF MS.

In this study we are evaluating the potential of SELDI-TOF MS as a diagnostic tool in Atlantic salmon (*Salmo salar*) to identify condition specific protein patterns in mucus and plasma, related to the effects of high-density stress. Fish (mean weight 160 g) were kept in fresh water tanks at three different high-density stress levels (100, 200, 300 kg/m^3), for 6 h. Control fish were kept at 22 kg/m^3. After the stress period water volume was adjusted to ensure that density level in all tanks was 22 kg/m^3. Samples of plasma and mucus were taken from 10 fish from each group during the post-stress period at intervals of 0, 60, 120, 180, 360, and 1440 min.

Standardisation of the mucus sampling technique is seen as an important prerequisite for successful profiling. Samples were collected by scraping both sides of the fish with an alcohol cleansed metal spatula. Areas of discolour mucus were avoided. Samples were immediately frozen in liquid nitrogen and stored at −80 °C. Following thawing, samples were prepared for further analysis by centrifuging at 12,000*g* at 4 °C for 20 min. The supernatant was collected and diluted 1:1 in a preparation buffer (50 mM Hepes pH 7.0, 0.05% Triton X-100, 10 mM DTT), and frozen in liquid nitrogen. The protein concentration was measured using the Bradford assay (Bradford, 1976) with the sample further diluted in binding buffer (50 mM sodium acetate buffer pH 4.35, 0.05% Triton X-100) to give a concentration of 0.4 mg/ml protein. Protease inhibitor cocktail (Sigma P8340) was added to give a final leupeptin concentration of 1.3 μM. Plasma samples were diluted 1:10 in binding buffer.

The protein chip array spots were pre-treated with binding buffer (2 × 200 μl per spot) for 5 min. The samples (both plasma and mucus) were then applied to the protein chip array (CM10 weak cationic exchanger chip) and incubated overnight with shaking at 4 °C. Following the incubation step the protein chip arrays were washed (3 × 200 μl per spot) with washing buffer (50 mM sodium acetate pH 4.35) and quickly rinsed with distilled water. Energy absorbing matrix (2 × 1 μl) was added to each spot, the matrix consisted of a saturated solution of sinapinic acid in 50% (v/v) acetonitrile and 0.5% trifluoracetic acid. The protein chip arrays were loaded into the protein chip reader. The protein chip arrays were analysed using a laser intensity of 170 to optimise for low molecular weight peptides/proteins (2–15 kDa) and with a laser intensity of 180 to optimise for higher molecular weight peptides/proteins (15–200 kDa). For each run an average of 105 laser shots were fired at each spot.

Examples of the spectra generated can be seen in Fig. 1. The spectra represent the sequentially recorded number of ions arriving at the detector (peak height/relative intensity) coupled with the corresponding mass/charge value. The mass spectra were calibrated using external mass calibrants, the data was also subjected to total ion current normalisation to minimise small differences in signal strength due to differences in protein concentration. Following the data preparation stage ''Biomarker wizard'' software (Ciphergen Biosystems) was used to collect the equivalent peaks present in a certain percentage of the experiment spectra into clusters. The software then returns a cluster summary with