Toxicoproteomics in aquatic toxicology: iTRAQ reveals insight into proteins affected by 17alpha-ethinylestradiol, dieldrin, and 17β-trenbolone

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Toxicoproteomics is an emerging discipline in toxicology for characterizing chemical modes of action at the molecular level. We have successfully utilized a quantitative proteomics method termed isobaric tagging for relative and absolute quantitation (iTRAQ) to measure protein responses to aquatic contaminants in the neuroendocrine brain (telencephalon and hypothalamus) and liver of two teleost species, the fathead minnow and largemouth bass. We typically detect between 200-600 proteins using LC MS/MS in fish tissues and are able to quantify between 10-20% because multiple, high quality peptides are detected from the precursor protein. False discovery rates generated from a reversed ray-finned fish database using both Proteomics System Performance Evaluation Pipeline (Protein Pilot, Applied Biosystems) and a second decoy database reveals that there is relatively high confidence in peptide-protein assignments (FDR = 5-10%). As expected, proteins identified in brain and liver are quite different. Many brain-specific proteins such as synaptosome-associated protein 25a, ependymin, glial fibrillary acidic protein, and brain-type fatty-acid binding protein are detected in complex protein mixtures of neuroendocrine tissues. Metabolic and structural proteins are abundant in both neuroendocrine and liver tissue and include GAPDH, aldolase, lactate dehydrogenase isoforms, beta-actin, and both alpha and beta tubulin. Perhaps most promising is that well characterized protein biomarkers for general and oxidative stress (e.g. heat shock proteins 70 and 90, glutathione-S-transferase, Cu/Zn superoxide dismutase, peroxidases) and markers for neurodegeneration (e.g. apolipoprotein E and microtubule associated protein Tau) can be quantified using quantitative proteomics. There are also fish specific proteins that are readily detectable with LC MS/MS that include the egg yolk precursor protein vitellogenin and ictacalcin which is located in chemosensory regions and the skin of fish. We present case studies using iTRAQ labeling to measure protein changes in response to an estrogenic pharmaceutical (17α-ethinylestradiol; EE_2), a neuroactive organochlorine pesticide (dieldrin), and an androgenic pharmaceutical (17β-trenbolone; Tb). These studies demonstrate that iTRAQ labeling methodology can be successful and informative in aquatic toxicology and can be used in non-model genomic species to better characterize a chemical mode of action.

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