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Determination of mercury fraction linked to protein of muscle tissue and liver of fish from Amazon region-Brazil

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Introduction

The fresh water fish fauna of the Amazon is considered the richest in the world, with more than 1.300 described species. This diversity of fish from different regions with in the Brazilian Amazon is still difficult to assess because the existing studies from several research institutes are sparse[1]. Commercial fishing is concentrated on approximately 40 exploited species, and among the most commonly caught are the following: dourada (Brachyplatystoma rousseauxii); pacu (Mylossoma sp, sp Myleus); jaraqui (Semaprochilodus spp.); tucunaré (Cichla spp.) and filhote (Brachyplathystoma filamentosum). These species represent 75% of the production of commercial fishing towns in the middle Madeira River of the Amazon in Brazil[2]. Gold mining in the Amazon region peaked in the 1980s/1990s, when an average of 200 tons of mercury (Hg) was released into the river and lake bottoms, and the Rio Madeira basin was one of the most affected areas[3]. Currently the construction of hydroelectric plants in Amazonian river basins has led to scientific discussions on the possible remobilization and bioavailability of mercury species that have been previously thought to be inert[4]. More than 75% of the mercury accumulated in freshwater fish tissue is found in its organic form, methylmercury (CH_3Hg^+), which is more likely to be absorbed from the water and diet than inorganic mercury[5]. When bio-accumulated in an organism, mercury can bind to metalloproteins, displacing atoms of elements essential for their proper function. The search of potential biomarkers for the identification and expression of metalloproteins bound to mercury may predict possible risks for fish contamination and human exposure due to changes in water quality in reservoirs.

Results and Discussion

This study utilized metalloproteomic techniques to characterize mercury-bound proteins in muscle and liver tissue of dourada and tucunaré collected in the AHE JIRAU - Madeira River basin-Brazil. The proteome of the muscle and liver tissue was obtained after two steps of fractional precipitation and the steps of the separating the proteins by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). Mercury was identified and quantified in the protein spots by graphite furnace atomic absorption spectrometry (GFAAS) after acid mineralization in an ultrasound bath[6]. GFAAS determinations indicated the presence of mercury in the protein spots with a molecular weight less than 20 kDa. The mercury concentrations in the spots in which this protein fraction was present were in the range of 11.40 – 35.10 μg kg^{-1}. Based on the mercury concentrations, it was possible to estimate that the protein spots contained approximately 1–3 mercury atoms per protein molecule and also presented stoichiometric ratios for mercury atoms per protein molecules. These protein spots were characterized by electrospray ionization tandem mass spectrometry (ESI-MS/MS) after trypsin digestion. From a total of 20 analyzed spots, 4 proteins showing Hg biomarker characteristics were identified:

PROTEIN	CHARACTERISTICS
Parvalbumin and its isoforms	this protein has divalent metal-binding sites available that can bind to Hg^{2+} ions
Ubiquitin-40S ribosomal protein S27a	zinc-finger metal binding domains. The zinc has many characteristics similar to those of mercury (weak acid)
Zinc finger and BTB domain containing protein 24	The presence of cysteine (weak base) in the peptide sequence may promote Hg^{2+} (weak acid) binding
Dual specificity protein phosphatase 22-B	

Conclusions

Using 2D-PAGE as a selective step in protein separation was confirmed to be very efficient, effectively separating many protein spots per gel. GFAAS could quantify the mercury present in the protein extracts, pellets and protein spots from both muscle and liver tissue. The identified protein spots by ESI-MS/MS that show biomarker characteristics can be used for monitoring, at a protein level, the toxic concentrations of mercury in fish species from the Amazon region.

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