In situ tissue analysis of metabolites, lipids, and proteins in the gill glands of Bloodfin Tetra by LAESI mass spectrometry

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INTRODUCTION

Mass spectrometric direct analysis of animal tissues is an emerging field enabled by atmospheric pressure ion sources. The adult males of Bloodfin Tetra (Aphyocharax anisitsi), a member of the Glandulocaudine subfamily of fish have modified caudal-fin scales associated with hypertrophied glandular tissue. No such glands are present in females. The functions of these glands are not established. The direct analysis of the exposed gill glands for metabolites, lipids and proteins could provide further insight into the functions of these structures. Laser ablation electrospray ionization (LAESI) mass spectrometry (MS) is known to simultaneously report on small metabolites, lipids and some proteins. In this contribution, we describe the in situ analysis of the tissue embedded gill glands by LAESI-MS.

METHODS

A. anisitsi were obtained locally from a pet store and each specimen was euthanized by submersion in buffered tricaine methanesulfonate for 10 minutes. The gill glands of A. anisitsi were surgically exposed. The entire fish was placed on a microscope slide attached to a 3-axis translation stage for sample maneuvering. The alignment of laser beam on the sample was aided by visualization using a CCD camera fitted with a macro lens. A Nd:YAG laser driven optical parametric oscillator (OPO) was utilized to produce mid-infrared pulse at 2940 nm for the ablation of the gill gland. A home-built electrospray intercepted and ionized the neutrals produced during ablation. The produced ions were detected by an orthogonal acceleration time-of-flight mass spectrometer.

PRELIMINARY DATA

LAESI mass spectra were collected from the exposed gill glands of the adult male specimens, from the tissue adjacent to the gill glands, and from the location corresponding to the gill glands in female specimens of A. anisitsi. The initial evaluation of the LAESI mass spectra from these tissues indicated presence of more than 200 positive ionic species in each local analysis. Over 120 small metabolites were found in the $m/z < 600$ range, lipid-related species were located in the $600 < m/z < 900$ range, and ~50 multiply charged peptide and protein peaks fell in the $1{,}000 < m/z < 2{,}000$ range. The structural identity of approximately 10% of these ions was determined based primarily on their tandem mass spectra. Examples of the identified small metabolites include choline, creatine, acetylcholine and histidine. The m/z range of 600 to 900 exhibits over 30 lipid peaks dominated by glycerophosphocholines, such as PC(34:2), PC(34:1), PC(36:2), PC(36:1), etc. In particular, the spectra of the gill glands in the sexually matured male showed the presence of multiply charged ions in the +6 to +10 charge states corresponding to a protein with a molecular weight of 11,386 Da. This protein was present in the male gill glands but absent in the adjacent areas of the male specimen and in the corresponding area of the female specimen. Most ambient ionization techniques exhibit relative sensitivities that favor a particular group of compounds. This phenomenon is usually attributed to ion suppression effects. The diversity of molecular classes detected in these spectra demonstrates that LAESI has the potential for the simultaneous detection of small metabolites, lipids and proteins. Identification of the protein uniquely associated with the male gill glands using rapid screening by LAESI technique requires further work based on conventional proteomics.

NOVEL ASPECT

LAESI-MS enables the simultaneous ambient analysis of metabolites, lipids and proteins locally in animal tissues.