The metabolomics of oxidative stress: Investigating the impact of a Sod1-null mutant and paraquat induced stress using Liquid Chromatography/Mass Spectrometry

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Introduction

Metabolism is a complex, inter-connected network of small molecule metabolites. Different mutations, stressors, or even species, can be expected to have characteristic features in the metabolome, i.e. metabolic profiles. Liquid Chromatography coupled to Mass spectrometry (LC-MS) is a powerful tool to quantify metabolites within an organism to help us understand biochemical and physiological processes. Here we describe a novel LC-MS protocol which we demonstrate to examine the metabolic profiles of two types of Drosophila: chemically or genetically derived, and the metabolic profile of four Drosophila species.

Methods

Adult male flies were homogenized in extraction buffer (3:1:1, methanol, chloroform, water). Proteins and debris were removed by centrifugation and the supernatant was brought to approximately 80% water for analysis on a microTOF-QQ mass spectrometer (Bruker Daltonics) with an ESI source coupled to a UltiMate 3000 rapid separation liquid chromatography system (Dionex). The LC was run with 10mM diammonium acetate at pH 4.95 using a watermethanol multi-step gradient on a Kinetex C18 100x2 mm, 1.5µm column (Phenomenex). The MS was run under negative ionization mode. Chromatograms were analyzed and molecular formula for unknown compounds were generated by SmartFormula using Database analysis software (Bruker Daltonics). T-tests were performed using MetaboAnalyst software (Bruker Daltonics) and heat maps were generated using metaanalyst 2.0.

Results

Nuclear molecules are notoriously difficult to separate with C-18 reversed phase columns and an alternative, hydrophilic interaction chromatography (HLIC), generates less than optimal peak shapes and reproducibility(2).

We have recently focused on establishing a C-18 reversed phase column based separation with a basic ion pairing reagent (IPR) to improve resolution. Fig 1 displays a series of polar standards we are currently able to resolve. Polar basic compounds are still poorly retained.

Genetic mutation: Superoxide dismutase–null (Sod-null) genotype

The SOD protein is a major component of the anti-oxidant system in cells(10). Loss of SOD function (Sod10 allele) results in many physiological changes including decreased lifespan(1). Our control is a transgenic rescue with 60% wild-type activity (See (5) for details). Here we demonstrate that loss of SOD function results in drastic metabolic rearrangements.

10% of metabolite features had different concentrations in the Sod-nulls and control lines (182 of 1818 features, P < 0.05).

Fig 2 shows a PCA scores and loadings plot representing the metabolic profiles of Sod-nulls and controls. Fig 3A shows the corresponding heatmap of the hierarchical clustering results. Sod-nulls and controls cluster separately. Within the Sod- and Sod+ groups there is sub-clustering especially within the S- flies. This grouping suggests variability in the biological response, as is expected for any biological system, and highlights the need for replication in the experimental design.

Fig 4A depicts three representative metabolites (molecular features) significantly (P < 0.05) changed between Sod-nulls and controls.

Glutamine is a precursor of glutathione (GSH), a major antioxidant component of biological systems, which reduces ROS and becomes an oxidized product (GSGG) – both are elevated in Sod-nulls vs. T5 Controls, B) Paraquat treated T5 lines vs. T5 unirradiated, and C) four Drosophila species.

Conclusions

We have developed an IPR-LC-MS protocol to detect and quantify a diverse set of polar metabolites and have successfully applied it to three experimental systems. This analysis has revealed new insights into the metabolic consequences of oxidant stress and the metabolic differences between Drosophila species.

The metabolic picture, however is incomplete and work is currently under way to identify a suite of currently unknown features using MS/MS analysis.

With a larger suite of known metabolites, biological implications of the three experimental parameters can be better understood. Future work will also focus on refining protocols and ESI positive mode analysis.