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Focused metabolic profiling of methylarginines and related biological amines

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Introduction: As our understanding of broad metabonomic analysis applied to human health and disease increases, there is an opportunity to utilize targeted metabonomics in order to identify biomarkers and uncover mechanisms of disease and drug toxicity. Of particular importance to diseases like cancer, cardiovascular disease and sepsis, are the metabolic pathways that encompass the biological network that links nitric oxide (NO) with amines, particularly the substrate for NO, L-arginine and the substrate-inhibitor asymmetric dimethylarginine (ADMA). In order to understand how L-arginine and ADMA function in health and disease, we need to be able to measure them efficiently, and there are several problems with this. Firstly NO, L-arginine and ADMA are difficult to measure in biological fluids. Secondly because of the opposing effects of arginine and methylarginines on NO release, it is important to measure both simultaneously. Finally, because the levels of arginine are regulated by other amino acids, particularly citrulline, glutamine and ornithine, it is important to consider a targeted array that includes these. Thus, using a 'focused metabolic profiling' approach, we aimed to develop a novel technique which measures L-arginine, methylarginines and a wide array of biological amines in one simple assay.

Methodology & Results: Ultra performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) was used to measure L-arginine and the methylarginines ADMA, monomethyl-L-arginine (L-NMMA) and symmetric dimethylarginine (SDMA) alongside over 30 other amines (Figure-1). Protein was removed from human or mouse samples (10 µl) using methanol. Samples were derivatised to improve resolution and mass spectrometric detection. MRM transitions were optimized through the infusion of standard compounds. MS analysis was performed using a XEVO tandem quadrupole mass spectrometer.

Conclusion: The focused metabolic profiling described above is the first world-wide study that provides a unique opportunity to identify novel mechanistic pathways and biomarkers involved in NO-related diseases such as cardiovascular disease.

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Insight into the biochemical, kinetic and spectroscopic characterization of garlic (*Allium sativum*) phytocystatin: Implication for cardiovascular disease

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Phytocystatins are cysteine proteinase inhibitors present in plants. They play crucial role in maintaining protease-anti protease balance and are involved in various endogenous processes. Thus, they are suitable and convenient targets for genetic engineering which makes their isolation and characterization from different sources the need of the hour. In the present study, a phytocystatin has been isolated from garlic (*Allium sativum*) by a simple two-step process using ammonium sulfate fractionation and gel filtration chromatography on Sephacryl S-100 HR with a fold purification of 152.6 and yield 48.9%. A single band on native gel electrophoresis confirms the homogeneity of the purified inhibitor. The molecular weight of the purified inhibitor was found to be 12.5 kDa as determined by SDS-PAGE and gel filtration chromatography. The garlic phytocystatin was found to be stable under broad range of pH (6-8) and temperature (30-60 °C). Kinetic studies suggest that garlic phytocystatins are reversible and non-competitive inhibitors are having highest affinity for papain followed by ficin and bromelain. UV and fluorescence spectroscopy revealed significant conformational change upon garlic phytocystatin-papain complex formation. Secondary structure analysis was performed using CD and FTIR. Garlic phytocystatin possess 33.9% alpha-helical content as assessed by CD spectroscopy.

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