

Characterization of DNA Adducts and Secondary Plant Metabolites at the Microgram Level using a Nanoelectrospray LC-MS-microcoil NMR Integrated System

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Novel Aspect:

LC-nanoelectrospray-MS combined with offline microcoil NMR for the characterization of minor DNA adducts and novel California poppy secondary metabolites.

Introduction

We recently reported an integrated high-throughput LC-MS-NMR platform for the characterization of mass-limited samples. The platform has high sensitivity for both MS and NMR, using nanoelectrospray MS and microcoil NMR offline, to accommodate the inherent differences between MS and NMR in their sample mass and time requirements. The platform is being applied to the characterization of DNA adducts being investigated as biomarkers for exposure to carcinogens, specifically in the analysis of the carcinogen aminobiphenyl (ABP) adducted to deoxyguanosine (dG). A second application is in the analysis of engineered cell cultures of the California poppy, which produce benzophenanthridine alkaloids (BPAs) that are of pharmaceutical interest, including the antimicrobial sanguinarine.

Methods

Normal-bore columns are required for high loading capacity for NMR. Nanospray MS, without loss of chromatographic resolution, is achieved with a novel nanoSplitter. Nearly all of the LC eluant is directed to a fraction collector for NMR analysis. Fractions are concentrated by drying and re-suspended in deuterated solvents before loading into the microcoil NMR using droplet microfluidics -- 3 μ L samples are pumped through Teflon capillary tubing separated by an immiscible fluorocarbon fluid.

DNA adducts were synthesized in a bio-mimetic reaction, in which metabolically activated deuterated and non-deuterated ABP was incubated with dG to generate dG-ABP adducts. Production of BPAs from the California poppy cell cultures was elicited by incubation with purified yeast extract and characterized using the LC-MS-NMR platform

Preliminary results

A new manual microdroplet method for loading microcoilNMR was developed for the DNA adducts, which provided faster set-up for single samples with interactive data acquisition. Additionally, this approach gave more efficient sample injections for trace compounds and more efficient recovery of the analyte after NMR, for follow-up analyses. Sample loading efficiency and recovery of the platform was evaluated by injection of 5mg dG standards into the LC-MS with fraction collection for NMR analysis. After NMR analysis, the sample was recovered and reanalyzed by LC-MS. Recovery was found to be 93% and the loading variance was determined to be within 1% RSD. The LOD for a 1D proton NMR spectrum was 250ng in a 1-hour acquisition of the reference compound

Indapamide. This set the target sample requirement; however, 50ng was sufficient when overnight acquisition was feasible.

The system was applied to the identification of dG-ABP adducts. The crude reaction mixture of the non-deuterated dG-ABP reaction yielded three chromatographically distinct compounds with the same MS and MS/MS fragmentation, suggesting they were isomeric species. The crude reaction mixture of the deuterated standards yielded 5 possible isomeric compounds. All compounds were collected and analyzed using microcoilNMR. The C-8-dG-ABP and C-8-dG-ABP-d9 were characterized by comparison of the NMR spectra with previously published data for the C-8-dG-ABP-monophosphate and by co-elution of the two species

A California poppy cell culture was challenged with yeast extract to elicit defensive alkaloid production. Separation of an extract with LC-UV (283 nm) detected 10 major metabolites. Six were tentatively identified from published work or comparison with standards. Work is ongoing to identify the unknown compounds and to confirm the known metabolites utilizing the automated LC-MS-microNMR platform.