Accurate Identification of Unknown and Known Metabolic Mixture Components by Combining 3D NMR with FT-ICR MS/MS

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Introduction

Metabolite identification in metabolomics samples is a key step that critically impacts downstream analysis. We recently introduced the SUMMIT NMR/MS hybrid approach for the identification of the molecular structure of unknown metabolites, based on the combination of NMR, mass spectrometry (MS), and combinatorial cheminformatics.

Experimental

For a mixture of metabolites, NHMFL's 9.4 T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) enabled resolution and identification of metabolite chemical formulas (Fig. 1, left). For each chemical formula, the possible molecular structures were generated, and their 1H and 13C NMR shifts calculated. Comparison to experimental two-dimensional 1H/13C chemical shift spectra then enables rank-ordering of the possible structures. (**Fig. 1**) The final structure may then be confirmed by FT-ICR MS/MS.

Results and Discussion

Here, we demonstrate the feasibility of the approach for an untargeted analysis of both a model mixture and an *E. coli* cell lysate, based on 2D/3D NMR experiments in combination with FT-ICR MS and MS/MS data. For 19 of the 25 model metabolites SUMMIT yielded complete structures that matched those in the mixture independent of database information. Of those, 7 top-ranked structures matched those in the mixture, and 4 of those were further validated by positive ion MS/MS. For 5 metabolites, not part of the 19 metabolites, correct molecular structural motifs could be identified. For *E. coli*, SUMMIT MS/NMR identified 20 previously known metabolites with 3 or more 1H spins independent of database information. Moreover, for 15 unknown metabolites, molecular structural fragments were determined consistent with their spin systems and chemical shifts. By providing structural information for entire metabolites or molecular fragments, SUMMIT MS/NMR greatly assists the targeted or untargeted analysis of complex mixtures of unknown compounds.

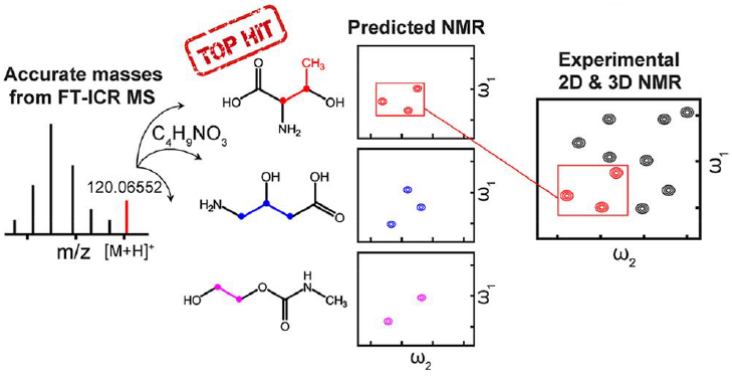

Fig. 1. Procedure for metabolite identification by combinin chemical formula from ultrahigh-resolution FT-ICR MS wit comparison of experimental and calculated two-dimensional NMR spectra (see text).

Acknowledgements

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Reference

[1] He, L., *et al.*, J. Proteome Res., **16**, 3774-3786 (2017).