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REVIEW

Two dimensional NMR spectroscopic approaches for exploring plant metabolome: A review

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A R T I C L E   I N F O

A B S T R A C T

Today, most investigations of the plant metabolome tend to be based on either nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS), with or without hyphenation with chromatography. Although less sensitive than MS, NMR provides a powerful complementary technique for the identification and quantification of metabolites in plant extracts. NMR spectroscopy, well appreciated by phytochemists as a particularly information-rich method, showed recent paradigm shift for the improving of metabolome(s) structural and functional characterization and for advancing the understanding of many biological processes. Furthermore, two dimensional NMR (2D NMR) experiments and the use of chemometric data analysis of NMR spectra have proven highly effective at identifying novel and known metabolites that correlate with changes in genotype or phenotype. In this review, we provide an overview of the development of NMR in the field of metabolomics with special focus on 2D NMR spectroscopic techniques and their applications in phytomedicines quality control analysis and drug discovery from natural sources, raising more attention at its potential to reduce the gap between the pace of natural products research and modern drug discovery demand.

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Introduction

NMR and plant secondary metabolites

The comparison of metabolite composition of biological systems (known as metabolomics) is now a mature field that has been increasingly applied to investigate a range of problems in plant and crop science [1,2]. A wide variety of analytical techniques have been employed in metabolomics, and each has its own advantages and drawbacks. The analytical techniques used to collect metabolomic data can be, broadly, split into two categories—those which separate the components of the crude solvent extracts prior to detection and those which directly analyze crude, unfractionated mixtures (detection is usually made by mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR)). Direct analysis by NMR is ideally suited to high-throughput metabolomics applications and has the advantage of detecting a wide range of metabolites in an inherently quantitative and unbiased manner. Compared to MS, NMR spectroscopy has a larger dynamic range for detection and is less biased since results of MS-based analyses greatly depend on choice of ionization conditions and the specific instrumentation used [3]. Albeit, NMR is less sensitive than other spectroscopic methods and can suffer from problems with signal overlap. The use of multidimensional NMR spectra can help in that regard by overcoming many of the problems encountered with one dimensional NMR and providing more detailed structural information [4].

One additional strength of NMR lies in its utility for the identification of unknown or unexpected compounds in a complex mixture. In initial plant metabolomics experiments, NMR use was mostly focused on the metabolic profiling of mixtures, and not yet being accepted as an appropriate tool for the definitive identification of novel or unexpected metabolites in a mixture. Only recently and driven in part by increases in NMR spectrometers sensitivity, extensive 2D NMR experiments and advances in data processing, NMR spectroscopic methods have begun to play a larger role in the identification of previously unknown small-molecules in complex mixtures. Such application is of great value in situations where some compounds are inaccessible, for example compounds that are prone to chemical decomposition and thus cannot be isolated [5]. Furthermore, it has become apparent that NMR spectroscopy-based metabolome analyses can be highly effective in identifying novel and known metabolites that correlate with changes in genotype or phenotype [6]. The present review provides the first overview on the advances made in the field of developing 2D NMR technologies to meet with applications in the field of plant metabolomics.

NMR spectroscopy: a historical perspective

Since its development in the middle of the past century, NMR has been an indispensable tool in the discovery of natural products largely replacing all traditional chemical degradation methods that were used for structural elucidation. Compared to other spectroscopic tools, NMR offers detailed structure information that can be surpassed only by X-ray crystallography while NMR remains much less demanding in terms of purity and sample preparation [7]. As a result, NMR spectrometers, despite their relative high cost, have become a core part in research laboratories and one of the main tools in natural product discovery. This wide spread use of NMR has led to fast improvement of both NMR hardware as well as supporting software. Advances in NMR spectroscopy have been remarkably accelerated during the past few decades driven, at least in part, by the demand to use NMR in the analysis of mixtures especially with the establishment of metabolomics as a new scientific discipline with myriad useful applications in both human and plant biology [7–10].

Since NMR spectroscopy measures the properties of nuclei and not molecules, response to NMR is uniform across all chemical classes and under certain experimental conditions, NMR enables absolute quantitation of metabolites through the integration of their corresponding ^{1}H NMR signals [11,12]. This method remains as the only acceptable approach to determine the concentration of plant chemical constituents in a crude extract without the need to use reference standard for each single constituent [13,14]. In addition to its value as a tool for metabolites quantification, NMR is a nondestructive technique from which the sample can be completely recovered for further analysis. The nondestructive nature of NMR as well as the minimal samples preparation for NMR acquisition poses this technique as being less prone to artifacts than other techniques commonly used in metabolomics. Moreover, with the introduction of autosamplers, ^{1}H NMR can be used as a high throughput technique since the acquisition time per sample is very short [15]. Nevertheless, the relative low sensitivity of NMR and the complexity of its generated spectra remain as the two main deterrents for wider application of NMR-based metabolomics.

Recent developments in NMR

The first NMR spectrometers were equipped with either electromagnets or permanent magnets and operated at a resonance frequency not higher than 60 MHz for proton [8]. Since then, sensitivity and resolution of NMR spectrometers have been greatly improved by the use of superconducting magnets that can operate at field resonance of up to 1 GHz [7,16]. Another major improvement in NMR