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NMR spectroscopy-based metabolomics: A review

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REVIEW

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ABSTRACT

A field of life science research that uses High Throughput (HT) technologies to identify and/or characterize all the small molecules or metabolites in a given cell, tissue or organism (i.e., the metabolome) is called metabolomics. The first NMR based metabolic studies were carried out by Wilson and Burlingame in the year 1974. NMR is one of the most selective analytical techniques which offer structural information of molecules.

The NMR workflow is as follows:

Sample preparation→Sample loading and spectral acquisition→Spectral processing→Spectral deconvolution→Data interpretation.

Future work is expected to build upon core strengths of NMR Spectroscopy, which includes its versatility and specificity in the form of 1D and higher dimensional spectra, its reproducibility, its quantitative ability, its capability for following chemical reactions and flux, its ability to identify compounds and deduce structures of unknowns, and its growing potential for collecting metabolomics data *in vivo*.

Key Words

Metabolomics, NMR, Chemical reactions

Metabolomics

Metabolomics is defined as the comprehensive analysis of metabolites i.e., identification and quantification in a biological system[3]. The metabolites identified by metabolomics are intermediary metabolites used to form macromolecular structures and other small molecules participating in metabolic functions and fulfilling critical roles such as signalling molecules and secondary metabolites. The study of small molecules (<1,500 Da) in living systems, provides information with a high potential for accurately describing the physiological state of an organism and this study can be achieved by metabolomics.

Approaches for the Analysis of Metabolome

The three principal approaches for the analysis of the metabolome:

1. Metabolite profiling
2. Metabolic fingerprinting
3. Metabolomics

Metabolite profiling:

Aims to identify and quantify metabolites, but does do on a biased scale due to methodological limitations and differences in analytical platforms[4].

Metabolite fingerprinting:

This high-throughput approach is normally utilized in tissue comparison or discrimination analysis, and so is simpler and coarser in its technique (sample preparation, separation, and detection) in comparison to metabolic profiling.

Metabolomics:

Focuses on the metabolic response of organisms to pathophysiological stimuli or genetic modification. This approach is generally restricted to microbiological and other non-botanical studies.

Measurement of the Metabolome

The two most successful approaches to determining the metabolic state of an organism are:

1. Mass spectrometry (MS)
2. Nuclear magnetic resonance (NMR) spectroscopy

Mass spectroscopy systems coupled with nuclear magnetic resonance systems are ideally the best platforms for identification of unknown chemical compounds, but are prohibitively expensive for most scientific laboratories

NMR Spectroscopy

Analytical chemistry is a technique used in quality control and research for determining the content and purity of a

sample as well as its molecular structure.

Principle Involved in NMR Spectroscopy

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level. The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

History of NMR Spectroscopy-Based Metabolomics Studies

Type 2 diabetes mellitus, Obstructive sleep, Family medicine[2]

1. 1974: First NMR based metabolic studies (Wilson and Burlingame)
2. 1977-1984: Extensive C13 and p31 NMR studies of cell systems (Shulman)
3. 1984: First NMR characterization of human urine (Nicholson)
4. 1986: The "Fossel" NMR blood test for cancer is described (ET Fossel)
5. 1991: NMR based HDL/LDL test developed (Otvos)
6. 1993: LC model software developed for in vivo NMR (Provencher)
7. 1999: The word "Metabonomics" was coined (Nicolson)
8. 2000: The word "Metabolomics" was coined (Drysdale)
9. 2006-2016: Large metabolomic projects (Wilhart, Markley)

The workflow pattern can be categorized into 3 steps:

Sample preparation

Treatment with methanol at solvent-to-serum ratio of 2:1 (v/v) - remove lipoproteins and minimize the loss of metabolites.

Silica nanoparticles – to remove protein

Tagging

NMR-active label is used to tag.

Ex: Nitrogen-15 with attached hydrogen is an attractive tag.

Quantification

Determination of absolute concentration by addition of standard of known concentration. Factors that translate peak intensity to concentration are determined. Peak intensities in spectra are converted into concentration whose intensities are proportional to concentration.

Applications of NMR Based Metabolomics

1. Disease diagnosis
2. Monitoring the effects of medical interventions including drugs, detection of adulteration of food, and analysis of biochemical pathways
3. Identification and quantification of co-enzymes that report on cellular function
4. To investigate alterations in the energy/redox-metabolome in dopaminergic cells exposed to environmental/mitochondrial toxins
5. In drug discovery programs to uncover the efficacy, specificity, or toxicity of lead compounds
6. *In vivo* mechanism of action and to eliminate compounds likely to cause problems with side effects
7. To search for biomarkers for colon cancer[5].

Future Considerations

1. The field needs to focus on developing standardized, enlarged, and integrated databases of NMR data.
2. Standardization of best practices for sample preparation, data collection and analysis should enhance the reproducibility of results within the metabolomics community.
3. The ideas of reproducibility and data sharing into every tool and database are to be built.

Advantages

1. NMR offers advantages for compounds that are difficult to ionize or require derivatization for MS.
2. NMR allows the identification of compounds with identical masses, including those with different isotopomer distributions.
3. For determining structures of unknown compounds.
4. Used to elucidate the dynamics and mechanisms of metabolite transformations and to explore the compartmentalization of metabolic pathways. NMR has advantages in drug screening[1].

Conclusion

Future work is expected to build upon core strengths of NMR spectroscopy, which includes its versatility and specificity in the form of 1D and higher dimensional spectra, its reproducibility, its quantitative ability, its capability for following chemical reactions and flux, its ability to identify compounds and deduce structures of unknowns, and its growing potential for collecting metabolomics data *in vivo*.

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