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NMR as a Progressive Tool for Bimolecular Studies: A Mini Review

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Abstract: Metabolomics is well recognized technique for the analysis of biological system. Recently NMR is becoming an important tool of interest for researchers in the area of bimolecular analysis, especially for drug discovery. This technique is a significant step forward for identifying biomarkers, ligand interaction to the target, pathway recognition and metabolomic mapping. NMR based metabolomic is not only being used for the identification and structure elucidation of synthetic or natural compounds but also is considered as an imperative tool for biomolecular drug discovery. Significant advancements in methodological developments, software (including bioinformatics tools), and hardware (including instrumentation) is exploring new areas for industrial drug discovery. In this review, we discussed the importance of NMR technique in bio-molecular sciences.

Key words: Bimolecular analysis, NMR, Metabolomics

Introduction

Metabolomics, as among other 'omic' strategies of systems biology, is the validation of metabolites in a biological system (Zhang et al., 2012). It is globally considered as an important tool for disease characterization, biomarker study and drug discovery (Smolinska et al., 2012). Among other metabolomic tools nuclear magnetic

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resonance (NMR) spectroscopy is being considered as promising tool for metabolomic characterization of drugs and toxic agents in body fluids (Engelke et al., 2007), furthermore it is the second important metabolomic tool for targeted and un-targeted metabolomic evaluation, after mass spectrometry (MS) (Smolinska et al., 2012). Even a single biological sample with number of variables affecting its metabolome, NMR generates a huge set of chemically diverse groups of metabolites in a single run (Ebbels, 2007). It is versatile technique that is being used, not only in academic to industrial research (both cases of ex vivo and in vivo), but also for routine analysis (Betz et al., 2006). Recently NMR is considered as amongst the most exciting analytical method in drug discovery, ranging from simple identification of a compound (or mixture of compounds) to complex structures of protein-ligand interactions (Roberts, 2000). Two dimensional NMR techniques are suitable to obtain information regarding structure of interactions of ligands and their targets. Furthermore, high throughput screening is obtained through automated screening by measuring 50 – 100 samples on daily basis for ligand–protein interactions. Whereas one dimensional techniques further allows screening of much high number of test compounds, per day, of low-molecular weight components in the test samples allow, even without less or no need of isotopic labeling (Ross and Senn, 2001).

Biomolecular studies provide dynamic and functional insights of active molecules. Although, more than 50 000 protein and nucleic acid structures has been solved by today, but still not yet enough insight has been developed so that the function of these biomolecular components could be predicted from structure or sequence alone. In this scenario further information is desirable about structure, dynamics, sequence and their functional understanding (Grzesiek and Sass, 2009).

Nuclear magnetic resonance (NMR) spectroscopy has been recognized as unique approach for the structure identification, quality assessment of drugs and also enabling to understand the mechanisms underlying for the process and/or degradation of impurities (Maggio et al., 2014). As a step further, there is a diversity of NMR applications for drug design from screening of natural leads to ligand specific binding of active compounds, used to discover and develop new therapeutic compounds (Stockman, 1998; Roberts, 2000). So NMR can contribute significantly in the drug development and manufacturing process (Fig. 1) (Aubin et al., 2015).

Methodology

NMR metabolic profiles are complex but information rich, having huge potential to look for the fundamental insights of molecular mechanisms underlying health and disease related tissues (Ebbels and Cavill, 2009). Effective collaboration among all stages of experimental design and interpretation of biological data is the basic point for the successful application of metabolomics (Ebbels and Cavill, 2009). Among various analytical methods for the research and development of drugs, NMR and MS is considered as a predominant method. These are not only being used for the structure elucidation of synthetic and/or natural products, identification of impurities (either drug degradation as by-products or contamination), while also it is used for the characterization of biomolecules, metabolomic studies, biomarker studies, drug–receptor binding sites, and structure–activity relationship studies (Görög and Szántay, 2010).

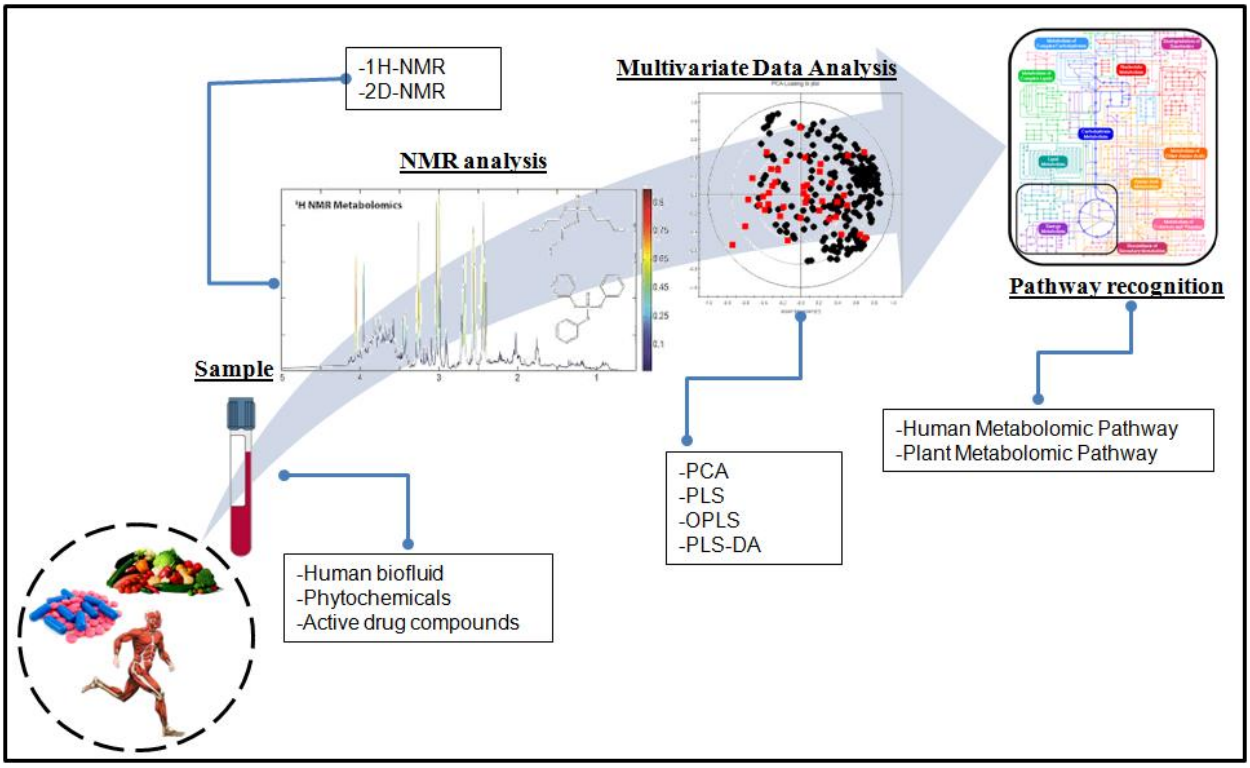

Figure 1. NMR as a progressive tool for bimolecular studies

Significant advancements have been made in NMR analysis of bio-molecules. Detailed structural information of biological systems can be produced through solid-state. Furthermore, biomolecules in the liquid system NMR and/or solid state NMR is used to produce significant information that can be used to design high-affinity ligands, by NMR screening. For this reason a number of NMR protocols have been developed to use both rational and random elements (Siegal et al., 1999). Among other techniques, detailed structural information and dynamics of biomolecules, can be obtained by magic-angle spinning solid-state NMR (Goldbourt, 2013). Methodological advancements for NMR analysis improved the precision and accuracy of results and extended the range of NMR application applicability to as a valuable component of the drug design (Roberts, 2000). Among other approaches in method development and standardization, the sample preparation and acquisition methods effects as discriminating factor for variability in NMR results (Smolinska et al., 2012). NMR is vastly regarded as non-destructive and quantitative method where biologically active compounds, such as amino acids and small peptides, steroids and flavonoids can be studied, providing valuable structural information (Paradowska & Wawer, 2014).

NMR-based ligand screening is widely recognized field of research. Further recent advancement in methodology and instrumentation have strengthened the applications of NMR based ligand screening, leading to further development of NMR as a tool for drug research in industry (Coles et al., 2003). The ligand and substrate-based fluorine NMR approaches are also known as the powerful tools for the screening of biomolecular targets. The NMR-based screening techniques have

advantages of reliability, robustness, reproducibility, stability and solubility of active compounds. Furthermore fluorine NMR-based screening is considered to have broad range of applications in drug discovery (Dalvit, 2007).

Additionally Isotope-filtered and isotope-edited NMR techniques have made considerable contributions for structure evaluation macromolecular complexes, and also to understand nature of biomolecular interactions for drug design. It will remain a discriminating factor in many of biomolecular investigations by NMR. Despite technological advancements in this field, NMR is not clear to provide complete high-resolution structures of very large systems (Breeze, 2000).

Bioinformatics

Bioinformatics cannot be estranged from challenges of biological metabolomic expedition (Ebbels & Cavill, 2009). Improvements in instrumentation, together with updates in bioinformatics extends the frontier of modern drug discovery (Betz et al., 2006). Analysis of NMR metabolomic data is usually attentive towards three main objectives, i.e. general visualization of overall differences (explaining association or disassociation between samples and variables), significance level of discrimination among the groups of interest, and identification of metabolites responsible for such discrimination. Furthermore, construction of prediction models of the biological samples is also matter of interest where multivariate data mining and analysis tools are the keys to achieve these goals (Ebbels, 2007). Despite of design of experiment and objective, comprehensive information can be obtained from NMR measurements, if adequately processed from ^{1}H-NMR, the time domain signal (FID), into a frequency domain spectrum. Further spectral processing i.e. bucketing, will enable NMR data to be processed for multivariate data analysis or to use for other bioinformatics evaluation (Čuperlović-Culf, 2013).

Biomarker discovery

Analysis of metabolites as a holistic approach for biological fluids (e.g. blood i.e. plasma /serum, cerebrospinal fluid, synovial fluid, urine, saliva, semen, and others) provides potential advantages to identify clinically important biomarkers that are correlated with disease (Zhang et al., 2012). This biomolecular correlation can be well investigated by NMR, that also provides a method for the synthesis and the fabrication of sugar-installed nanoparticles with tunable clustering ability, drug-loading efficiency, and controlled drug-release profile that is being used as drug delivery system (Dai et al., 2009).

Recently the capability of nuclear magnetic resonance (NMR) spectroscopy for biomarker discovery has increased with the advancement in instrumentation, specially increase in the sensitivity and bioinformatics resources for NMR data handling, processing and analysis have been matured (Van, 2013). NMR spectroscopic techniques are being used for biofluids evaluation to diagnose inborn errors of metabolism caused by single gene defects has been described. Metabolomic techniques to diagnose cardiovascular and cancer diseases have also been widely applied (Lindon & Holmes, 2007; Ebbels & Cavill, 2009). The investigation of biofluid composition and metaobolomic distributions as biomarker study, among living cells and tissues significantly impact the drug development for anticancer and/or neurological disorders (Betz et al., 2006).

Biomarker discovery by NMR combined with the pattern recognition techniques (including data acquisition and multivariate analysis) increasingly

contributes for uncovering disease mechanisms of complex neurological diseases (Smolinska et al., 2012). Additionally, isotopic labeling of active constituents of interest provides significant information for biomarker discovery and pattern recognition (Roberts, 2000).

Pathway recognition

Understanding of metabolic pathways is important to identify various metabolites that could be the drug targets and so considered as to be useful for the therapeutic research. NMR coupled with pattern recognition tools including multivariate data analysis (e.g. PCA, PLS-DA, OPLS-DA and others) can be integrated for the examination of metabolic signatures (Wang et al., 2012).The metabolite profiling, pathway recognition and network classification by NMR is relevant to other metabonomic profiling applications in biomolecular studies (Ott et al., 2003). NMR spectroscopy in combination with computational techniques provides the main source of structural information (Kövér et al., 2010). Solution NMR also provide structural insights into PCNA recognition to help for better understanding of TLS regulation in eukaryotes (Pustovalova et al., 2013). Metabolic pathways detection using correlative and quantitative NMR-based metabolomics approach is helpful to understand of disease-related mechanisms (Zhang et al., 2008). The pattern recognition and metabolomic pathway discovery for a diverse array of cell-wall components of Gram-negative and Gram-positive bacteria in the host inborn immune response by binding to pathogen-associated molecular patterns (PAMPs) at partially overlapping binding site(s) is examined by NMR spectroscopy (Albright et al., 2008). Furthermore, stable isotope-labeled precursors can be used in NMR spectroscopic analysis for the evaluation of biosynthetic pathways as well as to identify metabolite flux patterns (Bacher et al., 1998).

References

Albright, S., Chen, B., Holbrook, K.and Jain, N. U. 2008. Solution NMR studies provide structural basis for endotoxin pattern recognition by the innate immune receptor CD14. Biochem. Biophys. Res. Commun. 368: 231-237.

Aubin, Y., Freedberg, D. I. and Keire, D. A. 2015. Chapter 13 - One- and Two-Dimensional NMR Techniques for Biopharmaceuticals. In: Biophysical Characterization of Proteins in Developing Biopharmaceuticals. (Ed.): Berkowitz, D. J. H. A. Elsevier, Amsterdam, pp. 341-383.

Bacher, A., Rieder, C., Eichinger, D., Arigoni, D., Fuchs, G. and Eisenreich, W. 1998. Elucidation of novel biosynthetic pathways and metabolite flux patterns by retrobiosynthetic NMR analysis. FEMS Microbiol. Rev. 22: 567-598.

Betz, M., Saxena, K. and Schwalbe, H. 2006. Biomolecular NMR: a chaperone to drug discovery. Curr. Opin. Chem. Biol. 10: 219-225.

Breeze, A. L. 2000. Isotope-filtered NMR methods for the study of biomolecular structure and interactions. Progress in Nuclear Magnetic Resonance Spectroscopy, 36: 323-372.

Coles, M., Heller, M. and Kessler, H. 2003. NMR-based screening technologies. Drug Discov. Today, 8: 803-810.

Čuperlović-Culf, M. 2013. 4 - Metabolomics NMR data preprocessing – analysis of individual spectrum. In: NMR Metabolomics in Cancer Research. (Ed.): Čuperlović-Culf, M. Woodhead Publishing. pp. 215-259.

Dai, X.-H., Zhang , H.-D. and Dong, C.-M. 2009. Fabrication, biomolecular binding, in vitro drug release behavior of sugar-installed nanoparticles from star poly(ε -

caprolactone)/glycopolymer biohybrid with a dendrimer core. Polymer, 50: 4626-4634.

Dalvit, C. 2007. Ligand- and substrate-based 19F NMR screening: Principles and applications to drug discovery. Progress in Nuclear Magnetic Resonance Spectroscopy, 51: 243-271.

Ebbels, T. M. D. 2007. Chapter 7 - Non-linear Methods for the Analysis of Metabolic Profiles. In: The Handbook of Metabonomics and Metabolomics. (Eds.): Lindon, J.C., Nicholson, J.K., Holmes, E. Elsevier Science B.V., Amsterdam. pp. 201-226.

Ebbels, T. M. D. and Cavill, R. 2009. Bioinformatic methods in NMR-based metabolic profiling. Progress in Nuclear Magnetic Resonance Spectroscopy, 55: 361-374.

Engelke, U. F. H., Oostendorp, M. and Wevers, R. A. 2007. Chapter 14 - NMR Spectroscopy of Body Fluids as a Metabolomics Approach to Inborn Errors of Metabolism. In: The Handbook of Metabonomics and Metabolomics. (Eds.): Lindon, J.C., Nicholson, J. K., Holmes, E. Elsevier Science B.V., Amsterdam. pp. 375-412.

Goldbourt, A. 2013. Biomolecular magic-angle spinning solid-state NMR: recent methods and applications. Curr. Opin. Biotechnol. 24: 705-715.

Görög, S. and Szántay, J. C.. 2010. Spectroscopic Methods in Drug Quality Control and Development. In: Encyclopedia of Spectroscopy and Spectrometry (Second Edition). (Ed.): Lindon, J. C. Academic Press, Oxford. pp. 2640-2650.

Grzesiek, S. and Sass, H.-J. 2009. From biomolecular structure to functional understanding: new NMR developments narrow the gap. Curr. Opin. Struct. Biol. 19: 585-595.

Kövér, K. E., Szilágyi, L., Batta, G., Uhrín, D. and Jiménez-Barbero, J. 2010. 9.07 - Biomolecular Recognition by Oligosaccharides and Glycopeptides: The NMR Point of View. In: Comprehensive Natural Products II. (Eds.): Liu, H.-W., Mander, L. Elsevier, Oxford. pp. 197-246.

Lindon, J. C. and Holmes, E. 2007. Chapter 15 - A Survey of Metabonomics Approaches for Disease Characterisation. In: The Handbook of Metabonomics and Metabolomics. (Eds.): Lindon, J. C., Nicholson, J. K. and Holmes, E.. Elsevier Science B.V., Amsterdam. pp. 413-442.

Maggio, R. M., Calvo, N. L., Vignaduzzo, S. E. and Kaufman, T. S. 2014. Pharmaceutical impurities and degradation products: Uses and applications of NMR techniques. J. Pharm. Biomed. Anal. doi: 10.1016/j.jpba.2014.04.016.

Ott, K.-H., Aranı, Amp, Bar, N., Singh, B. and Stockton, G. W. 2003. Metabonomics classifies pathways affected by bioactive compounds. Artificial neural network classification of NMR spectra of plant extracts. Phytochemistry, 62: 971-985.

Paradowska, K. and Wawer, I. 2014. Solid-state NMR in the analysis of drugs and naturally occurring materials. J. Pharm. Biomed. Anal. 93: 27-42.

Pustovalova, Y., Maciejewski, M. W.and Korzhnev, D. M. 2013. NMR Mapping of PCNA Interaction with Translesion Synthesis DNA Polymerase Rev1 Mediated by Rev1-BRCT Domain. J. Mol. Biol. 425: 3091-3105.

Roberts, G. C. K. 2000. Applications of NMR in drug discovery. Drug Discov. Today, 5: 230-240.

Ross, A. and Senn, H. 2001. Automation of measurements and data evaluation in biomolecular NMR screening. Drug Discov. Today, 6: 583-593.

Siegal, G., Van Duynhoven, J. and Baldus, M. 1999. Biomolecular NMR: recent advances in liquids, solids and screening. Curr. Opin. Chem. Biol. 3: 530-536.

Smolinska, A., Blanchet, L., Buydens, L. M. C.and Wijmenga, S. S.2012. NMR and

pattern recognition methods in metabolomics: From data acquisition to biomarker discovery: A review. Anal. Chim. Acta, 750: 82-97.

Stockman, B. J. 1998. NMR spectroscopy as a tool for structure-based drug design. Progress in Nuclear Magnetic Resonance Spectroscopy 33: 109-151.

Van, Q. N. 2013. Chapter 6 - Current NMR Strategies for Biomarker Discovery. In: Proteomic and Metabolomic Approaches to Biomarker Discovery. (Ed.): Veenstra, H. J. I. D. Academic Press, Boston, pp. 87-117.

Wang, X., Yang, B., Zhang, A., Sun, H. and Yan, G. 2012. Potential drug targets on insomnia and intervention effects of Jujuboside A through metabolic pathway analysis as revealed by UPLC/ESI-SYNAPT-HDMS coupled with pattern recognition approach. Journal of Proteomics, 75: 1411-1427.

Zhang, A., Sun, H.,. Wang, P., Han, Y. and Wang, X. 2012. Recent and potential developments of biofluid analyses in metabolomics. Journal of Proteomics, 75: 1079-1088.

Zhang, S., Nagana Gowda, G. A., Asiago, V., Shanaiah, N., Barbas, C. and Raftery, D. 2008. Correlative and quantitative 1H NMR-based metabolomics reveals specific metabolic pathway disturbances in diabetic rats. Anal. Biochem. 383: 76-84.