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G. A. WEBB

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Preface

Volume 61 of Annual Reports on NMR consists of a collection of five reviews pointing to the importance of NMR studies in many areas of scientific research. It begins with an account of Resolution Enhancement in *in vivo* NMR Spectroscopy by C. Faber; this is followed by a discussion of Spin Echo NMR Diffusion Studies from F. Stallmach and P. Galvosas; Recent Advances in Theoretical Calculations of Indirect Spin–Spin Coupling Constants are covered by L. B. Krivdin and R. H. Contreras; Q. Chen and H. Kurosu review Solid-State NMR Studies on Semicrystalline Polymers; finally J. Farjon, L. Ziani, L. Beguin, D. Merlet and J. Courtieu report on Selective NMR Excitations in Chiral Analysis.

It is my pleasure to express my gratitude to all of these authors for their timely and interesting accounts of the recent research developments in the diverse areas of NMR involvement covered in this volume. My thanks also go to the production staff at Elsevier for their assistance in the regular production of volumes of Annual Reports on NMR.

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October 2006

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Resolution Enhancement in *In Vivo* NMR Spectroscopy

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In vivo NMR spectroscopy requires adequate spectral, spatial, and temporal resolution. Current methodology provides numerous efficient methods to optimize all three kinds of resolution. The achievable spatial and temporal resolution mainly depend on the experimental setup including the object that is studied, the magnetic field strength, and the hardware used for signal detection. Spectral resolution is a much more sensitive parameter. While the maximum resolution is also limited by the experimental setup, small and apparently unimportant influences can dramatically deteriorate spectral resolution. This chapter shortly reviews current methodology and limits in spatial and temporal resolution in in vivo NMR spectroscopy. Dipolar fields causing inhomogeneity of the magnetic field, which often leads to severe line broadening, are discussed as major nuisance to spectral resolution. Several methods to avoid or refocus line broadening are discussed. Shimming and susceptibility matching are methods that reduce field inhomogeneities in the sample. Two-dimensional spectroscopy can provide resolution of frequency differences that are smaller than the actual line widths. Two techniques are discussed that use physical mechanisms to actively refocus line broadening. Magic angle spinning averages out dipolar interactions, while distant dipolar field (DDF) spectroscopy,

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also termed intermolecular zero-quantum coherence (iZQC) spectroscopy, uses the local nature of the DDF to locally refocus magnetization. The origin of the DDF is discussed in detail and a pictorial explanation of signal refocusing is given. Current DDF spectroscopy methods and their in vivo applications are summarized.

1. INTRODUCTION

In vivo NMR spectroscopy (MRS) has the same fundamental objective as any high-resolution NMR experiment: resolving spectral patterns, which are the main source of information that can be obtained. Chemical shift, fine structure, and peak intensities constitute a 'fingerprint' of every molecule, allowing for its identification and for quantification in multicomponent samples, including living organisms. Multiplicity patterns and integrated peak areas yield information on molecular structure. Chemical-shift values provide structural information and its variations over time indicate reaction kinetics. For small molecules in solution, analysis of chemical shift and fine structure of every atomic nucleus is often no problem in a modern high-resolution spectrometer. Even in large molecules such as proteins the full spectroscopic information is accessible. There, severe overlap of resonance lines is avoided by the analysis of multidimensional experiments using hydrogen, carbon, and nitrogen nuclei to achieve sufficient dispersion of chemical shifts. First prerequisite for every high-resolution NMR experiment is to have extremely homogeneous magnetic fields inside the probe. For molecules in solution in susceptibility-matched glass tubes, this precondition can be achieved efficiently with elaborate shim units, which are part of every modern NMR spectrometer. However, if the sample itself is not homogeneous, as for instance in emulsions, resins, specimens of tissue or rock, or in living organisms, resolution may be heavily compromised. For the application to living organisms many efficient remedies for different aspects of this problem have been demonstrated over the last decades. *In vivo* NMR spectroscopy has become a highly developed and valuable tool in medical diagnostics and biomedical research with animal models for numerous diseases.[1–4] MRS provides unique advantages compared to other diagnostic techniques. It allows collecting data from tissue inside the living organism. The penetration depth MR can '*look inside the body*' is virtually unlimited; an advantage only afforded by nuclear medicine techniques PET and SPECT. These, however, require application of marker substrates that are radioactive. Other techniques, such as fluorescent imaging have strongly limited penetration depth and also require application of marker substances. MRS combines the advantages of being absolutely noninvasive, providing unlimited penetration depth, and not requiring marker substances.

In principle, MRS can detect any isotope with a nonzero nuclear spin. Practical limitations are, of course, given by their abundance in the organism. Therefore, the most relevant nuclei that are observed by *in vivo* MRS are ^{1}H, ^{13}C, and ^{31}P. Two other nuclei involved in metabolic events have been omitted here. ^{17}O and ^{23}Na are often detected by MR spectroscopic methods, yielding important physiological information. ^{17}O-labeled water content, produced from inhaled ^{17}O gas, allows for

quantification of oxygen use in the organism.[5] ^{23}Na is the naturally abundant sodium isotope and its concentration is indicative for the function of the cellular sodium potassium pump. Strong increase in the detected sodium concentration can be related to a breakdown of the pump and is therefore a marker for tissue viability.[6,7] However, total signal intensities of both ^{17}O and ^{23}Na are normally quantified and related to physiological function. Although measured with spectroscopic techniques no further spectral information is exploited, making such investigations more imaging than spectroscopy.

^{1}H is part of almost every metabolically relevant molecule. Its high gyromagnetic ratio makes *in vivo* metabolite detection at concentration below 1 mM possible.[2] Information on many important neurotransmitters and carbohydrate metabolites, for example *N*-acetyl aspartate (NAA), creatine (Cr), choline (Cho), lactate, glutamine/glutamate (Glx), glucose, myo-inositol (mI), γ-amino butyric acid (GABA), aspartate (Asp), and taurine (Tau) can be provided by ^{1}H MRS. Fig. 1 shows an exemplary spectrum obtained at a magnetic field strength of 17.6 T from a 5–mm voxel in the rat brain *in vivo*. A number of metabolites are detectable and can be quantified from spectra of such quality.

^{13}C MRS has the advantage of large chemical-shift dispersion resulting in very good spectral resolution. The smaller gyromagnetic ratio and the low natural abundance of the ^{13}C isotope (1.1%) hamper detection of metabolites not present at high concentrations. If patients or animals are infused or fed with ^{13}C-labeled substrates, for instance glucose, turnover rates of metabolic reactions can be determined.[8,9]

Phosphorous MRS provides good spectral dispersion and the lower gyromagnetic ratio is compensated by the high natural abundance of ^{31}P. High-energy

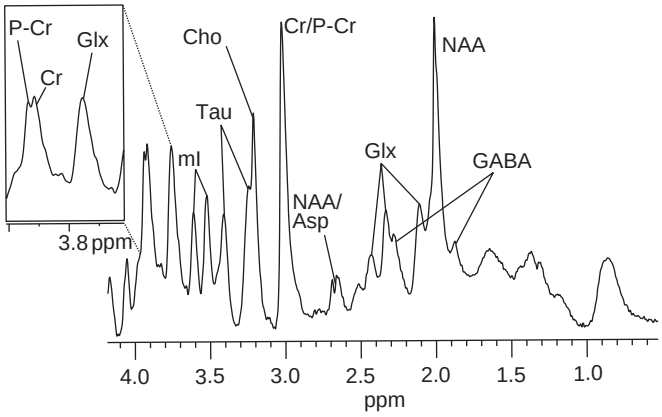

Fig. 1. *In vivo* ^{1}H single voxel MRS from the rat brain at 17.6 T (spectrum courtesy of Thomas Neuberger). *In vivo* ^{1}H spectrum from a (5 mm)^3 voxel positioned in the center of the brain of a female Fisher rat. The resonances were assigned according to Ref. 12 and referenced relative to the residual water signal, which was set to 4.7 ppm. Prior to acquisition shimming of an (8 mm)^3 voxel was performed using FASTMAP. There were 128 averages collected in 9 min scan time. A number of metabolite resonances were observed with narrow line widths, resolving the Cr and P–Cr lines at 3.9 ppm.

phosphate metabolites such as ATP and phosphocreatine are directly observable. Intracellular pH and intracellular magnesium concentration can be determined, making ^{31}P MRS a valuable tool for the study of numerous diseases.[9,10] Independent of the observed nucleus, if a distinct spectral line is resolved, quantification or relative intensities can be directly linked to biochemical, metabolic, or physiological events.[1] Deviations from normal values can be used as indicators for pathologies and thus for detection and characterization of diseases. Kinetics of metabolic or biochemical reactions can be studied, if spectra are acquired in a temporally resolved manner.

In vivo MRS is particularly powerful for applications in large organs without pronounced intrinsic structure. Traditionally it is used for investigations of the brain. Numerous studies have demonstrated that unique information on tumors, damage after hypoxia, or a number of neurodegenerative diseases can be obtained.[2–4] Localized ^{1}H NMR spectra from the mouse brain have been used to obtain cerebral metabolite profiles of different mouse strains.[11] A study at a magnetic field strength of 9.4 T measured 18 different metabolites in the rat brain, composing a neurochemical profile.[12] Recently, quantification of Vitamin C, as 19th metabolite, was accomplished in a similar setup.[13] Proton MRS has also been applied successfully for investigations of the spinal cord, despite the problems imposed by its smaller size and strong respiratory and cardiac motion.[14–17] In cardiovascular research MRS methodology is highly developed for investigation of related diseases. Proton spectra have been recorded from isolated hearts of different organisms including mice,[18] rats,[19] and rabbits.[20] *In vivo* ^{1}H MRS has been performed successfully in dogs,[21] humans,[22] and mice[23,24] Efficient shimming procedures and effective gating strategies were crucial to avoid artifacts imposed by cardiac and respiratory motion, blood flow, and differences in magnetic susceptibility in the nearby lung.[22,25] Fig. 2 exemplifies the spectral quality that can be achieved in the mouse heart, if *state-of-the-art* methods for motion compensation and shimming are applied. Major cardiac metabolites were observed and could be quantified (Fig. 2a). Metabolic dysfunctions, as for example in genetically modified mice, can be detected (Fig. 2b). Further applications of MRS to skeletal muscle, the liver, or other organs have been reported. However, intrinsically inhomogeneous regions are problematic and compromise the diagnostic potential. Objects such as the lung, leaves of green plants, or tissue near air interfaces or near metallic implants are inaccessible with MRS.

This chapter gives an overview of the requirements to obtain spatially and spectrally resolved MR spectra from living organisms. Since the physical and experimental conditions influencing resolution of a spectrum are similar for different nuclei, the focus will be on ^{1}H MRS. Most of the considerations are also valid for any other nucleus. Concrete solutions, however, may not always be compatible. The following sections start with a classification into spatial, temporal, and spectral resolution. Basic conditions encountered in *in vivo* MRS and requirements to obtain spatially and temporally resolved spectra are discussed. Conventional methodology is reviewed shortly. The focus of this chapter is on spectral resolution. Magnetic field inhomogeneities as major source of line broadening are explored.

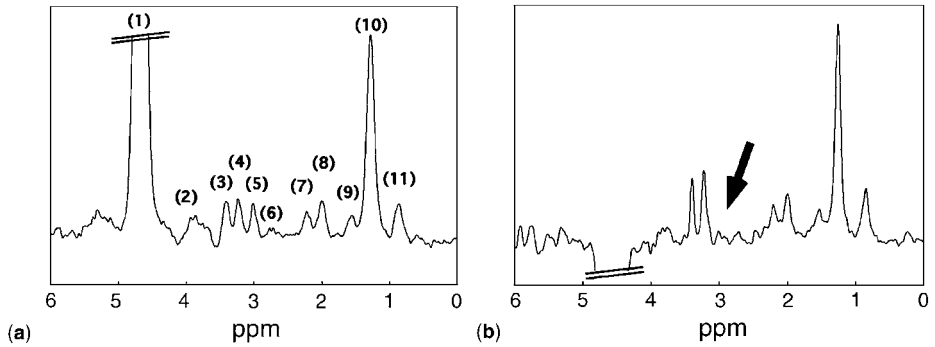

Fig. 2. *In vivo* cardiac ^{1}H single voxel MRS in mice at 11.75 T (from Ref. 24 with permission). (a) ^{1}H spectrum from a 2 µl voxel positioned in the interventricular septum of a wild type mouse *in vivo*. The resonances were assigned according to Ref. 19 and referenced relative to the residual water signal (peak (1)), which was set to 4.7 ppm; (2) (P)Cr–CH_2, 3.88 ppm; (3) taurine, 3.38 ppm; (4) carnitine, 3.21 ppm; (5) (P)Cr–CH_3, 2.99 ppm; (6) unassigned, 2.72 ppm; (7) glycerides (CH), 2.20 ppm; (8) unassigned, 2.0 ppm; (9) glycerides (CH), 1.55 ppm; (10) glycerides (–CH_2)*n*, 1.26 ppm; (11) glycerides terminal methyl, 0.85 ppm. (b) Spectrum from a guanidinoacetate *N*-methyltransferase deficient mouse (GAMT−/−), where no creatine was detectable (black arrow). Both spectra consisted of 512 averages and were scaled equally.

Subsequently, several techniques to reduce line broadening are discussed. Spectroscopic techniques using the distant dipolar field (DDF) are treated in detail, explaining the underlying mechanisms and reviewing current applications.

2. RESOLUTION IN *IN VIVO* MRS

In vivo MRS requires a more differentiated definition of resolution than is the case for high-resolution NMR, where the term 'resolution' clearly refers to spectral resolution. There, the samples are usually isotropic and duration of the measurement is irrelevant, unless slow kinetic processes are directly observed. *In vivo*, spectral resolution is only one aspect, besides temporal and spatial resolution. Measurement time is strictly limited by the time a subject can tolerate inside the magnet, an animal can be kept under anesthesia, or in extreme cases a plant needs to grow out of the probe. This defines a time scale during which any experiment has to be completed. More important for experimental considerations, if metabolic processes are studied, their time scale defines the temporal resolution required.

Spatial resolution is the aspect of resolution that is most particular to *in vivo* MRS. Metabolic information, obtainable from the spectrum, is desired from only one or a limited number of well-defined regions in the organism under investigation. Most studies focus on one organ requiring localization strategies that limit the detected signal to the region of interest. Furthermore, information on the local