In vivo $^{19}$F NMR chemical-shift imaging of Ancistrocladus species

G. Bringmann, K. Wolf, M. Meininger, M. Rokitta, and A. Haase

1 Lehrstuhl für Organische Chemie I and 2 Lehrstuhl für Experimentelle Physik V, Universität Würzburg, Würzburg

Received March 23, 2001
Accepted July 6, 2001

Dedicated to Professor Manfred Christi on the occasion of his 60th birthday

Summary. $^{19}$F nuclear magnetic resonance (NMR) imaging and $^{19}$F NMR chemical-shift imaging ($^{19}$F CSI) have been used to localize fluorinated compounds administered to stems of Ancistrocladus heynanus and A. abbreviatus for the elucidation of biosynthetic pathways in living plants. This first application of $^{19}$F CSI on plants proved CSI to be a valuable technique for mapping fluorinated molecules in plants. Exemplarily using trifluoroacetate as a model compound allowed to select appropriate feeding methods and to optimize both concentration and duration of the application to the plant. The time course of the uptake and distribution of trifluoroacetate was monitored by both $^{19}$F imaging and $^{19}$F CSI. Fluorinated metabolites formed by uptake of 3-fluoro-3-deoxy-D-glucose were detected with $^{19}$F CSI.

Keywords: Fluorine nuclear magnetic resonance imaging; Fluorine chemical-shift magnetic resonance imaging; Ancistrocladaceae; Trifluoroacetate; 3-Fluoro-3-deoxy-D-glucose; In vivo nuclear magnetic resonance.

Abbreviations: 3-FDG 3-fluoro-3-deoxy-D-glucose; CSI chemical-shift imaging; NMR nuclear magnetic resonance; SNR signal-to-noise ratio; TFA trifluoroacetate.

Introduction

In vivo nuclear magnetic resonance (NMR) methods, well established in medical and pharmacological research, gain importance in investigations of plants (Chudek and Hunter 1997, Ratcliffe 1994). The non-invasive character of these techniques enables the acquisition of NMR data from living plants revealing anatomical and physiological parameters like water distribution (Connelly et al. 1987, Glidewell et al. 1999, Kuchenbrod et al. 1995, Mc Fall and Johnson 1994) or flow velocities (Rokitta et al. 1999a, b). Biochemically relevant data are obtained by monitoring the distribution of primary and secondary metabolites (Heidenreich et al. 1998; Meininger et al. 1997; Metzler et al. 1994, 1995; Wolf et al. 2000; Ziegler et al. 1996). The overwhelming majority of published work concentrates on the ubiquitous $^1$H nucleus carrying the NMR information (Chudek and Hunter 1997, Ratcliffe 1994). In particular, metabolic studies, by contrast, are often based on nuclei like $^{13}$C, $^{19}$F, or $^{31}$P. Due to a wider range of chemical-shift differences, their NMR spectra exhibit less signal overlap than in vivo proton NMR spectra, where interpretation often fails because of overlapping signals from different protons in similar environment. Moreover, heteronuclei can be used for more specific investigations as their natural occurrence is relatively poor, allowing spectroscopical detection of administered compounds with almost no disturbing background signals. Furthermore, the NMR-active isotope $^{19}$F is present at 100% natural abundance, a very favorable situation compared to carbon, where only 1% of its naturally occurring isotopes consists of the NMR-active $^{13}$C nucleus and 99% is NMR-invisible $^{12}$C, necessitating enrichment processes or long measuring times for acquiring carbon NMR signals. Combined with the very high NMR sensitivity of 83% compared to the $^1$H nucleus, fluorine bears ideal properties for permitting the successful application of $^{19}$F NMR spectroscopy and imaging for the detection of fluorine-labelled substances (Berkowitz et al. 1990) and for the use of fluorinated compounds as NMR contrast agents (Wyrwicz et al. 1983). In spite of these apparent ben-
enefits, only three applications of $^{19}$F NMR imaging on plants have as yet been published (Rollins et al. 1989, Rowland et al. 1993, Wyrwicz et al. 1986).

Tropical lianas of the plant family Ancistrocladaceae produce naphthylisoquinoline alkaloids (Bringmann and Pokorny 1995) from acetate units (Bringmann et al. 2000). The intermediates of this biosynthesis are yet unknown. Feeding biosynthetic precursors in a fluorine-labelled form to living plants and NMR detection of the marker in the metabolites subsequently formed seems a promising approach. The purpose of our study was thus to apply conventional NMR imaging and chemical-shift imaging (CSI) as analytical methods both on the nucleus $^{19}$F and on members of the liana genus Ancistrocladus in order to investigate the limits of these techniques for a possible elucidation of biosynthetic pathways in living plants.

Previous work had revealed the plant Ancistrocladus heyneanus to be suitable for $^1$H NMR in vivo investigations (Meininger et al. 1997a, b). In general, NMR imaging methods (Callaghan 1991, Gadian 1995) enable to image the spatial distribution of one sort of nuclei with a defined chemical shift. Two-dimensional methods like spin-echo or gradient-echo imaging excite an overall NMR signal in one selected cross-sectional slice of the investigated object. The emerging signal is encoded in two spatial dimensions by use of magnetic field gradients. Final procession of the spatially encoded signal by Fourier transformation gives the NMR image. Different chemical shifts present within one sort of nuclei lead to chemical-shift artifacts within the image. CSI (Brown et al. 1982, Maudsley et al. 1983, Pohmann et al. 1997) as a three-dimensional method discriminates between spatial and spectroscopic information (by phase encoding in two spatial directions) and avoids these artifacts. With one spectroscopic and two spatial dimensions, CSI yields a spectrum of each volume element (voxel), permitting the localization of distinct NMR signals and thus the generation of metabolite maps.

In this paper, we report on the optimization of both imaging methods using the fluorine nuclei by feeding of the model compound sodium trifluoroacetate (TFA) to A. abbreviatus and A. heyneanus. The distribution of the fluorinated compound in dependence of the concentration of the feeding solution and of the time was studied on A. heyneanus. On A. abbreviatus, the time course of such an experiment was monitored by $^{19}$F imaging and $^{19}$F CSI. The uptake and metabolic fate of 3-fluoro-3-deoxy-D-glucose (3-FDG) in A. heyneanus was monitored by $^{19}$F CSI to test CSI for biosynthetic investigations on plants by metabolite mapping.

Material and methods

Ancistrocladus heyneanus and A. abbreviatus plants were grown from mature seeds. Both were cultivated in a tropical greenhouse of the Botanical Garden of the University of Würzburg. Detailed information on the cultivation conditions has been given by Bringmann et al. (1993, 1999).

NMR studies were carried out on approximately 1-year-old plants of A. heyneanus and A. abbreviatus. The different parts of the plants (selected leaves, roots and 1–5 cm of freshly cut ends of the shoots) were immersed in glass tubes or flasks filled with different concentrations of aqueous sodium TFA (1.0, 0.1, and 0.025 M) or 0.1 M 3-fluoro-3-deoxy-D-glucose (3-FDG) solutions. For detailed explanation of technical equipment, principles of NMR imaging methods, and data processing, refer to the textbooks of Callaghan [1991] and Gadian [1995].

The experiments were performed on a Bruker Biospec 70/20 NMR spectrometer with a magnetic field strength of 7 T and a horizontal bore of 20 cm (Bruker Analytische Messtechnik GmbH, Rheinstetten, Federal Republic of Germany). The actively shielded gradient coils were capable of achieving gradients of up to 200 G/cm with rise and fall times of less than 200 μs. A homemade Helmholz-type radio frequency coil with a diameter of 1.0 cm, tunable to both the $^1$H and $^{19}$F resonance frequency (300.3 and 282.5 MHz, respectively), was used for excitation and detection of NMR signals.

For $^1$H and $^{19}$F imaging, a two-dimensional cross-sectional NMR spin-echo sequence was used. The image matrix sizes were 128 by 128 picture elements for the proton image and 32 by 32 picture elements for the fluorine image, thus requiring 128 and 32 single measurements for each average, respectively. Typical parameters were: echo time, 7.8 ms; repetition time, 1.0 s; sweep width, 25 kHz; field of view, 5 by 5 and 10 by 10 mm; resolution, 43 μm ($^1$H) and 140–310 μm ($^{19}$F); slice thickness, 4–10 mm.

The three-dimensional $^{19}$F CSI experiment consists of a spin-echo sequence. To ensure short echo times at typical acquisition times of 100 ms, only half of the echo was detected. The maximum echo time was 5.9 ms. Phase-encoding gradients in both directions were applied simultaneously with the spoiler gradient before the 180° pulse, and 961 single acquisitions were recorded for one complete CSI data set. Baseline and phase balance artifacts were suppressed using the Exorcycle phase cycle. Typical parameters were: echo time, 6.1 ms; repetition time, 1.0 s; sweep width, 12, 15, and 25 kHz; field of view, 5 by 5 and 10 by 10 mm; resolution, 140–310 μm ($^{19}$F); slice thickness, 4–10 mm.

$^{19}$F CSI data were zero-filled in spatial direction to 32 points and phase corrected. Exponential filtering was done in spectroscopic direction and Hanning filtering in spatial direction. After three-dimensional Fourier transformation, the real part of the spectra was phase corrected. Processing was done on IBM RS-6000 workstations (IBM, Stuttgart, Federal Republic of Germany) with Interactive Data Language (IDL Research Systems, Boulder, Colo., U.S.A.) routines written by our group.