PROTON DETECTED VOLUME-SELECTIVE ^{13}C SPECTROSCOPY AND TOMOGRAPHY *IN VIVO*

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Abstract

The sensitivity of ^{13}C magnetic resonance spectroscopy and imaging can considerably be enhanced by selectively detecting the hydrogen nuclei bound to the ^{13}C nuclei of interest. The maximum signal enhancement theoretically is a factor of 64 times the number of protons coupled to the ^{13}C nucleus. Pulse sequences are reported which permit indirect ^{13}C imaging as well as image-guided volume-selective editing of signals of hydrogens bound to ^{13}C. The methods are designed to be partially insensitive to the pulse phases and amplitudes and, as far as possible, to motions. The sequences have been implemented on 4.7 T and 2 T NMR tomographs. Tests have been carried out with phantom samples as well as with human volunteers.

Volume-selective spectroscopy

Previously (1) we have suggested a heteronuclear volume-selective single-scan editing (heteronuclear VOSING) technique using the split-pathway principle. The improved techniques to be reported now are based on multiple quantum filtering (MQF) and cyclic polarization transfer (CYCLPOT) in addition to the split pathway (SP) principle again (2). The signals are edited as coherence and spin state transfer echoes (2,3). The CYCLPOT-VOSING pulse sequence (fig.1) consists of three slice-selective 90° proton pulses (P_1,P_2,P_3), producing an echo C.
The editing principle is the CYCLPOT method (5). The CYCLPOT sequence starts with an excitation of protons followed by a transfer of polarisation to carbon with a selective ^{13}C-pulse. After the evolution of coherences in the carbons and transfer back to protons follows the detection of the edited ^1H-signal. The pulse sequence is "phase-insensitive", meaning that only the phases of all initial preparation pulses and the reference phase of the instrument have fixed values, while the phases of all other RF pulses are arbitrary. This means, the sequence is easy to adjust.

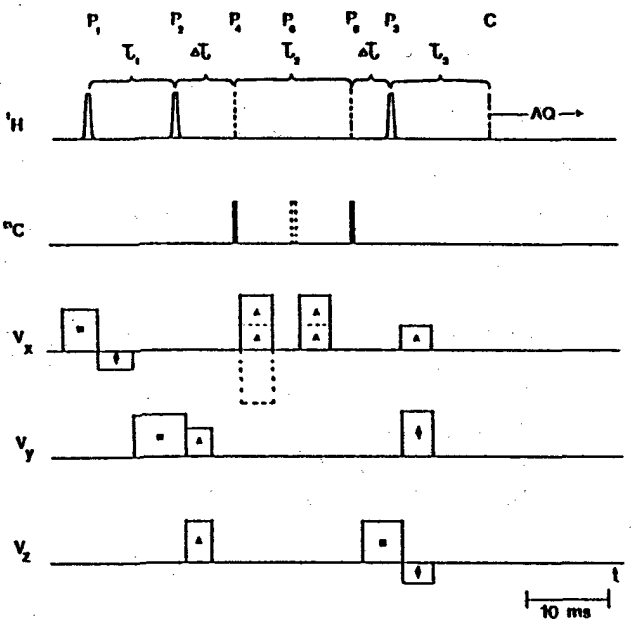

fig. 1: RF and field-gradient pulse sequence of the heteronuclear CYCLPOT-VOSING technique.

Multiple-quantum filter VOSING spectroscopy is carried out by the aid of four RF pulses only. The selection of the volume element is provided by three slice selective 90° pulses, which produce a volumeselective coherence transfer echo. In contrast to the homonuclear VOSY sequence (4), the slice selection pulses are applied in different frequency channels.

In split-pathway VOSING spectroscopy, a split-pathway echo is produced selectively for the protons coupled to ^{13}C. A subsequent pulse transfers the magnetization corresponding to the split-pathway echo in the z direction. Coherences of uncoupled spins are not affected by this pulse so that their coherences can be spoilt in the subsequent pulse interval.
All three methods are single-scan techniques. This means there is no subtraction of FID's recorded at different scans. Therefore the methods are less sensitive to movements. The accumulation serves only the S/N-improvement and phase cyclization to avoid undesired coherences.

Test spectra

The methods were tested on a BRUKER 4.7 T-BIOSPEC Tomograph with a sphere of 1 cm diameter, filled with ^{13}C-enriched methanol. In spectrum (fig. 2a), detected with the VOSY technique without editing, the singlet of the hydroxyl protons of alcohol and water appears at 4.7 ppm. The doublet of methyl protons coupled to ^{13}C is centered at 3.2 ppm.
With the VOSING technique (spectrum b and c) only the doublet of methyl protons is produced and the hydroxyl protons are almost completely suppressed without any presaturation of water.
Another series of experiments has been carried out with a 2 T whole-body tomograph. Figure 3 shows the spectra recorded from the subcutaneous tissue of the calf of human leg. The VOSY spectrum (fig. 3a) shows the signals of lipid methyl and methylene protons in the region between 1 and 2.5 ppm. The water signal at 4.7 ppm was presaturated and has therefore a disturbed lineshape. The small signal at 5.7 ppm is assigned to the unsaturated protons of the fatty acids. In spectrum (b) the ^{13}C doublet of saturated fatty acid protons is detected by the aid of a selective ^{13}C pulse at 30 ppm. All other resonances are suppressed very well. In spectrum (d) the selective ^{13}C pulse was irradiated at 130 ppm, the frequency of the unsaturated fatty acid carbons. Now the signals of the unsaturated protons are selectively detected. But the high-field doublet line is obscured by the residual signal of the presaturated water line. Remarkably, with the VOSING sequence (fig. 3d) the scarcely visible peak at 5.7 ppm in the VOSY spectrum (fig. 3a) was magnified to form the strongest signal.

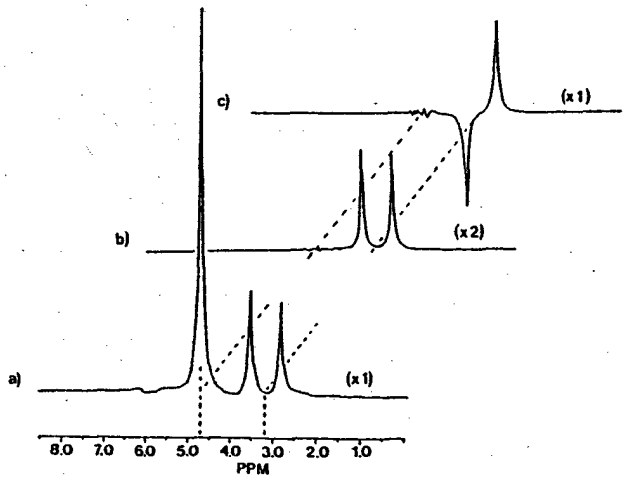

fig. 2: Comparison of volume-selective spectra recorded from an aqueous solution of ^{13}C enriched methanol with the 4.7 T instrument. All spectra have been acquired in one scan only from a volume element of $(1\ cm)^3$ of the phantom sample.
a) Homonuclear proton VOSY in the "low-coupling limit" without specific editing measures. The spectrum is dominated by the water line (at 4.7 ppm) and by the ^{13}C doublet of the methyl group centered at 3.2 ppm.
b) CYCLPOT-VOSING spectrum.
c) MQF-VOSING spectrum.

In another experiment we applied the method to the fat of the tibial bone marrow of a volunteer. The selected volume element ($3*1*1\ cm^3$) implied a tubular section of the bone marrow of corresponding dimensions. Difficulties arose from the small volume of the bone marrow and the great difference in the susceptibility between bone marrow, bone and surrounding muscle. The editing technique obviously works satisfactorily even under such complicated circumstances (fig. 4).

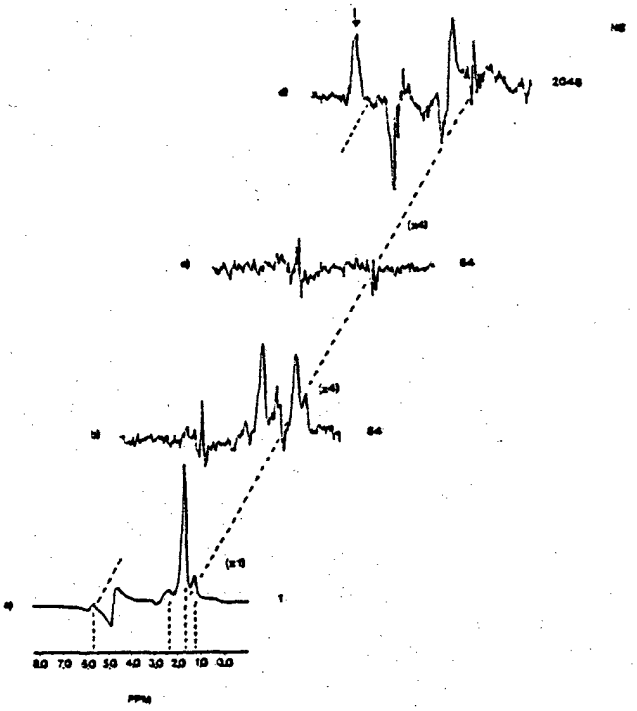

fig. 3: Comparison of volume-selective 2 T spectra recorded from subcutaneous adipose tissue of the calf of a test person. Volume of interest: 3*1*3 cm^3.
a) Homonuclear proton VOSY without specific editing measures.
b) CYCLPOT-VOSING spectrum. P_4 and P_5 have been irradiated at 30 ppm (saturated fatty acids).
c) CYCLPOT-VOSING spectrum with ^{13}C pulses turned off. Practically no signal is visible demonstrating the quality of the editing effect.
d) Same as b) , but P_4 and P_5 have now been irradiated at 130 ppm (unsaturated fatty acid groups). Only the left satellite line is visible (arrow), the right satellite line is superimposed by the residual waterline.

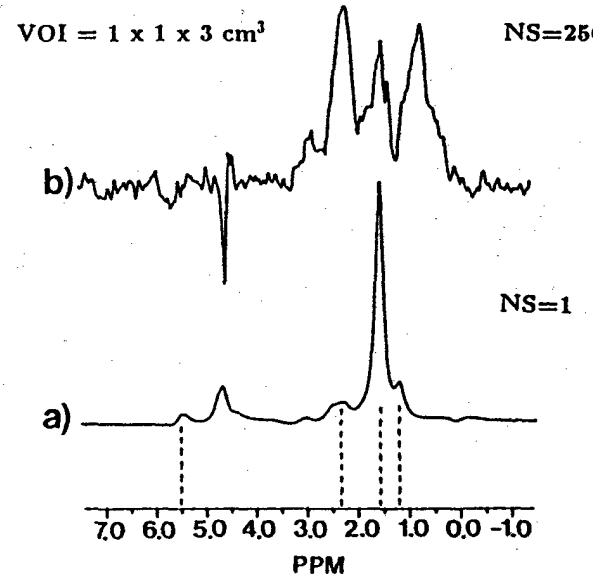

fig. 4: Comparison of volume-selective 2T spectra recorded of lipid in human tibial bone. a) Homonuclear proton VOSY spectrum without specific editing measures. The spectrum is dominated by the lipid methylene line.
b) CYCLPOT-VOSING spectrum with the edited lipid methylene doublet.

Imaging

The volume-selective editing sequences can also be combined with conventional Fourier transform imaging methods (6,7), so that proton images reflecting the distribution of ^{13}C nuclei are recorded.
With the technique described in the following, ^{13}C images can indirectly be recorded with proton sensitivity using the heteronuclear multiple-quantum filter editing method (fig. 5) (8). The pulse sequence for hydrogen/carbon tomography (HYCAT) produces the coherence-transfer echoes C of protons coupled to ^{13}C nuclei while signals of uncoupled protons are suppressed. The coherence transfer signal is recorded in the presence of a self-refocusing reading gradient. If necessary, the pulses P_2 and P_3 can be shaped to act selectively on a desired ^{13}C line. The second dimension is scanned by phase encoding gradients applied in x direction and incremented with positive and negative

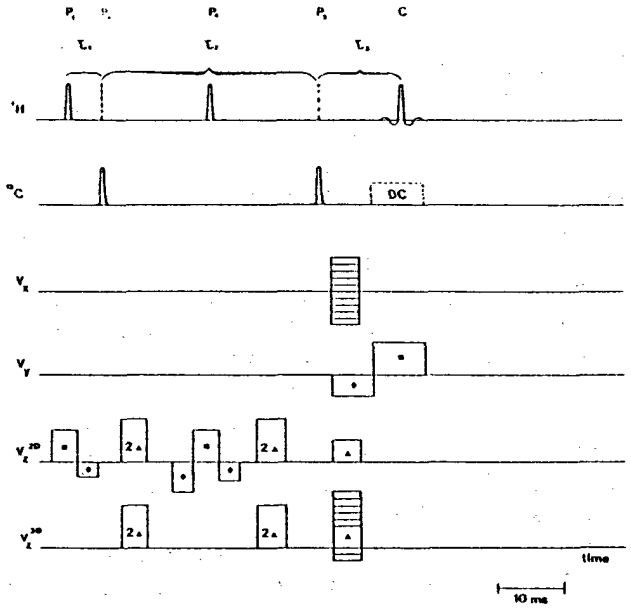

fig. 5: RF and field-gradient pulse sequences for two- or three-dimensional ^{13}C imaging by proton resonance (HYCAT). The control voltages V_x, V_y, V_z for the field-gradient pulses are drawn in scale. For the z gradients, alternative sequences for two- (V_z2D) or three-dimensional (V_z3D) imaging are given.

sign . The selection of the slice to be imaged in the two-dimensional version is carried out by the aid of slice-selective "soft" pulses in the presence of field gradients. The gradient pulses are self-refocusing. In the case of three-dimensional imaging, the third space direction is also scanned by phase encoding gradients incremented in z direction.

Test Images

The pulse sequence was tested by the aid of a sphere, 4 cm in diameter, filled with methanol with ^{13}C in natural abundance. The sphere was placed in a beaker filled with water. The regular, not edited proton image shows both methanol sphere and water cylinder with different intensities depending on different proton densities.
The ^{13}C edited HYCAT image shows the methanol sphere only. Note that the complete suppression of the surrounding water is an editing effect specific to the HYCAT method, because no water presaturation was applied (fig. 6).

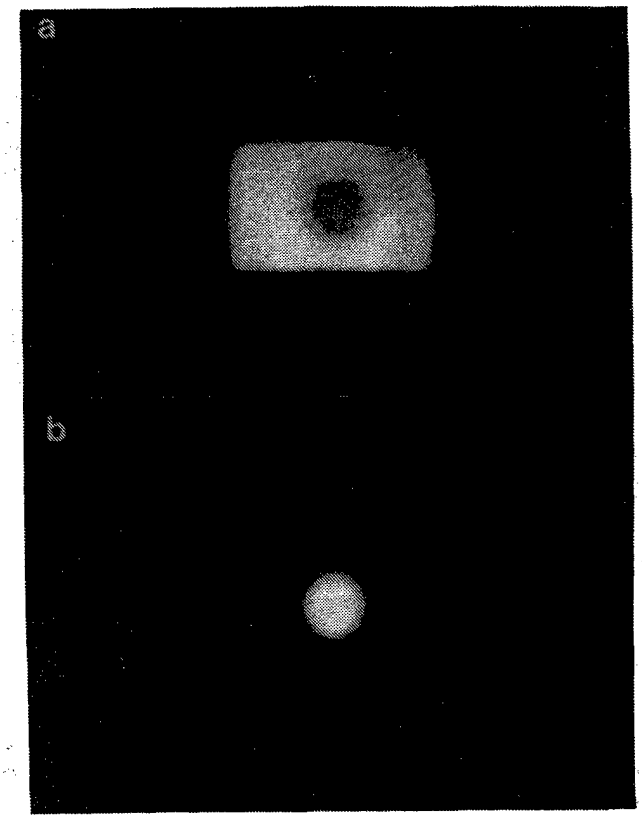

fig. 6: Images of the phantom sample (methanol sphere positioned in water).
a) was recorded with the normal two-dimensional spin-echo technique without editing but otherwise the same adjustments as in b).
The image b) was acquired by the aid of the two-dimensional version of the HYCAT sequence. The slice thickness was 20 mm, the number of accumulation scans 16. No intensity of the water region is visible anymore.

As a biological object, a fresh shank of veal was imaged at 4.7 T. The signals of muscle are strongly reduced compared with those of bone marrow (published elsewhere) (8).
In a whole-body 2 T tomograph a normal sagittal proton image of a human knee with a slice thickness of 5 mm was recorded (fig. 7). It shows a good resolution of anatomical details.
If the slice thickness is increased to 15 mm, the image becomes more blurred and details like ligaments and menisci disappear.

fig. 7: Images of a knee of a volunteer.
a) was recorded with the normal twodimensional spin-echo technique without editing but otherwise the same adjustments as in c), slice thickness 5 mm.
b) was recorded with the same adjustments as in a) but with a slice thickness of 15 mm.
Image c) was acquired with the twodimensional version of the HYCAT sequence. The slice thickness was 15 mm again.

The indirect ^{13}C-image (^{13}C in natural abundance) measured with the HYCAT-method of the same 15 mm slice of the knee has nearly the same anatomical resolution.

It should be mentioned that the achieveable spatial resolution and therefore the resolution of anatomical details is in principle equivalent to that of conventional proton imaging, because the slice detection, the phase encoding and the read gradients are applied while proton pulses or coherences are relevant. The limitation is given by the sensitivity and the available measuring time. A great improvement in sensitivity will be achieved by application of ^{13}C enrichend substances. As a further option the region of interest can be made visible selectively.

Conclusions

The indirect detection of ^{13}C containing compounds by proton resonance is the most efficient and sensitive procedure for ^{13}C NMR spectroscopy. The maximum enhancement compared with direct ^{13}C detection theoretically is a factor of 64 times the number of protons coupled to a ^{13}C nucleus. Simultaneously one has optimal measuring conditions: A combination of high sensitivity with great structural resolution and short repetition times because of the lower spinlattice relaxation times of the protons. No decoupling is required as in regular ^{13}C spectroscopy. Therefore the RF dose absorbed in tissue is low. The three pulse sequences for volume-selective spectroscopy have proven to work quite reliably. Only minor advantages or disadvantages with respect to each other can be stated. The three pulse sequences are single scan methods. This means subtraction of signals detected in different scans is not necessary. Therefore only minimal movement artefacts are expected. The accumulation serves only S/N improvement and phase cycling. The phase-insensitivity of the pulse sequences moreover guarantees ease of handling and adjustment.
The combination of two- or three-dimensional Fourier transform imaging techniques with the heteronuclear editing techniques permit the record of ^{13}C images with an optimal sensitivity: The initial excitation as well as the signal detection is carried out with protons coupled to ^{13}C nuclei. The selection of compounds occurs via carbons.
Under such conditions it should be possible to image the distribution of ^{13}C enriched drugs or metabolites. ^{13}C is an ideal tracer because there is no perceptible influence on biochemical processes as it can be expected for ^{2}H as a label nucleus, for instance.

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