Two-Dimensional Zero-Quantum Coherence ^{1}H NMR Spectroscopy of Glutamate and Glutamine

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Introduction:

In vivo detection of the important neurometabolites glutamate (Glu) and glutamine (Gln) by means of NMR spectroscopy remains a challenge, not only due to their high metabolic turnover, but also to complex scalar-coupled multiplets and strong signal overlap in the ^{1}H spectrum. In order to more precisely differentiate the two, we make use of zero-quantum coherences (ZQC) in a stimulated-echo acquisition mode (STEAM) sequence. This is based on the method of volume-localized editing by means of ZQC in a STEAM sequence as introduced by Sotak and Freeman [1]. By varying the mixing-time interval (TM) between the second and third RF pulses of the STEAM sequence, phase modulation of scalar-coupled spins can be observed. The frequency of this modulation is equal to the difference of the chemical shifts of the coupled spins. Addition of a second axis along which the frequency of the ZQC evolution is plotted, allowed detection of lactate in human brain tumor *in vivo* at B_0 = 1.5 T in a measurement time of 31 min. [2].

For Glu with its α-CH peak at δ = 3.74 ppm and β-CH peak at δ = 2.03 ppm, the frequency of the ZQC modulation is 212 Hz for the outer α-CH triplet peaks at B_0 = 3T. For Gln at the same B_0, this value is 203 Hz due to Gln's α-CH peak at δ = 3.75 ppm and β-CH peak at δ = 2.13 ppm. [3].

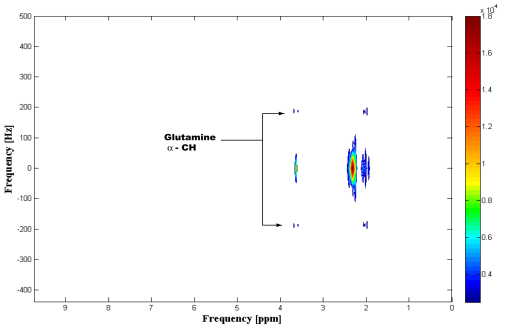

Fig. 1 2D ZQC ^{1}H NMR spectrum of Gln model solution (40 mmol/L) showing Gln α-CH peaks. TR = 2000 ms, TE = 85 ms, starting TM = 10 ms, ΔTM = 1 ms, nex = 64, 16 steps, voxel = 2x2x2 cm^3, measurement time = 34 min.

Materials and Methods:

Glutamate and glutamine model solutions (40 mmol/L each) were examined via localized two-dimensional (2D) ZQC ^{1}H NMR spectroscopy on a whole-body MR tomograph (3 T, Magnetom Trio; Siemens Medical Solutions, Erlangen, Germany). Series of water-suppressed ^{1}H NMR spectra were obtained from a 2x2x2 cm^3 voxel with TR = 2000 ms. Series of sixteen spectra were recorded from the phantoms with nex = 64 (4 prescans before the first spectrum only), TM starting at 10 ms and increasing in steps of ΔTM = 1 ms, and TE varying from 61 to 77 ms and 103 to 113 ms for Glu and 68 to 90 ms for Gln for different series. This resulted in a total measurement time of 34 minutes per series. Additionally, a series of 32 spectra was recorded from a model solution containing NAA, choline chloride, creatine hydrate, myo-inositol, lithium lactate, and Glu at their respective *in vivo* brain concentrations (brain model). Parameters were the same as in the case of the Glu and Gln model spectra. TE was set to 72 ms. All spectra were zero-filled and apodized with a 5-Hz Lorentzian filter before further evaluation.

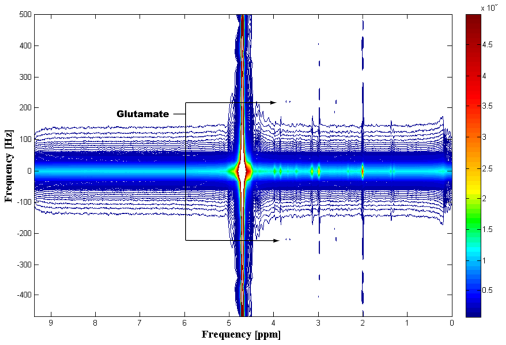

Fig. 2 2D ZQC ^{1}H NMR spectrum of brain model solution (*in vivo* concentrations) showing Glu α-CH peaks. Measurement parameters as in Fig. 1, except TE = 72 ms, 32 steps, measurement time = 68 min.

Results:

Based on the *J*-coupling constants of 7.33 and 4.65 Hz for the Glu α-CH with the β- and β'-CHs respectively, and 6.50 and 5.85 Hz for the Gln α-CH with the β- and β'- CHs respectively [3], one expects the optimal TE times for generation of ZQC to be 68 and 111 ms for Glu and 77 and 85 ms for Gln (TE = 1/(2*J*)). Although this could be verified for Gln with optimal TE ranges between 73 and 77 ms and 85 and 86 ms, the best TE times for Glu were found to be in the range of 63 to 65 ms. Figure 1 shows Gln measured at TE = 85 ms. Figure 2 shows the brain model measured at TE = 72 ms. In both cases, the x-axis shows the chemical shift (in ppm) and the y-axis the ZQC evolution frequency (in Hz). The intensity of the peaks is indicated with color-coded contour lines. The outer peaks of the α-CH triplet of Gln in the Gln model solution (a) and Glu in the brain model (b) could be clearly identified due to their chemical shifts and ZQC modulation. In Fig. 1, the multiplets from the β- and γ-CHs are also visible (the water has been removed via a Hankel Lanczos Singular Values Decomposition (HLSVD) filter). In Fig. 2, the strong water peak as well as the peaks of the other metabolites can be seen. In Fig. 3, the intensity of the cross-spectra at 0 Hz (red) and 219 Hz (blue) along the ZQC modulation axis from Fig. 2 is plotted over the chemical shift axis. The 0 Hz cross-spectrum corresponds to a non-ZQC modulated, i. e. the conventional ^{1}H STEAM spectrum. In the blue spectrum, which is taken at the ZQC modulation frequency, the outer Glu α-CH peaks can be easily recognized. This is due to their ZQC modulation and the singlets lack thereof.

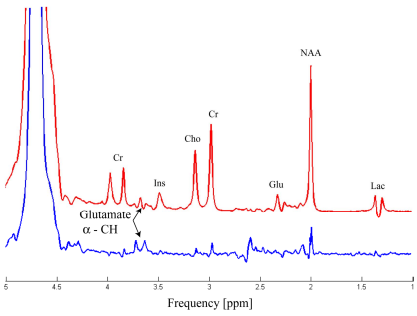

Fig. 3 Intensity of slices (1D spectra) through 2D spectrum in Fig. 2. Red: slice at 0 Hz; blue (intensity multiplied with a factor of 10): slice at 219 Hz, which corresponds to the ZQC modulation frequency of Glu.

Dicussion:

We have proven that both Glu and Gln can be well identified in individual solutions due to their ZQC modulation frequency, as can Glu in solution with other metabolites. In spite of this, initial attempts to resolve a model solution containing both Glu and Gln prove more challenging due to the high resolution that is necessary along the ZQC frequency axis. We conclude, that were it possible to increase the number of TM increments without increasing the measurement time, Glu and Gln could be unequivocally differentiated.

References:

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[3] V. Govindaraju, V. J. Basus *et al*. Magn. Reson. Med. **39**: 1011-1013 (1998).