**17O Quadrupole Central Transition NMR at 35.2 T**

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**Introduction**

Oxygen is among the most common elements in biological molecules, but remains to be a challenging one to study directly by NMR spectroscopy, despite the fact that considerable progress has been made in the past decade [1,2]. One useful approach known as quadrupole central transition (QCT) NMR makes it possible to obtain relatively narrow signals for half-integer quadrupolar nuclei such as 17O (*I* = 5/2) under the ultraslow motion condition in the liquid state [3]. We have previously shown that 17O QCT can be used to study biological macromolecules as large as 240 kDa in aqueous solution [3]. Since the line width of 17O QCT signals generally decreases with the applied magnetic field, the installation of a series-connected hybrid magnet at 35.2 T at the NHMFL represents an excellent opportunity to further explore the resolution limit in 17O QCT NMR spectroscopy.

**Experimental**

17O QCT experiments were performed on the series-connected hybrid (SCH) magnet at 35.2 T and 28.1 T. A homebuilt single-resonance 3.2 mm MAS probe was used, which is designed for quadrupolar and low-gamma nuclei NMR experiments with a wide tuning range. 17O QCT experiments at 18.8 T were performed on the 800 MHz 63 mm superconducting magnet using a 3.2 mm homebuilt mid-bore MAS probe. Single pulse experiments were conducted for [3,5,6-17O3]-D-glucose glycerol solution at various temperatures from 255 to 305 K. A liquid sample of H2O was used for both radio frequency (RF) field strength calibration and for 17O chemical shift referencing at 276 K at all magnetic field strengths. The excitation pulse used for 17O QCT NMR experiments has a duration of approximately one-third of that for an effective 90° pulse for the liquid H2O sample.

QCT-Figure-1**Results and Discussion**

**Fig.1** shows 17O QCT NMR spectra of [3,5,6-17O3]-D-glucose in glycerol solution recorded at two magnetic fields. At 18.8 T, the full width at the half height (FWHH) for the 5-O signal is about 18 ppm. At 35.2 T, however, this value is reduced to less than 5 ppm. With this drastic line width reduction at 35.2 T, all three 17O QCT NMR signals arising from 5-O, 3-OH, and 6-OH groups are well resolved. It is well known that the carbohydrate molecules such as glucose represent a great challenge to 17O NMR because all the oxygen-containing functional groups in this class of molecules exhibit large 17O quadrupolar coupling constants (ca. 9-12 MHz) but very similar chemical shifts. As a result, it is usually not possible to obtain resolved 17O NMR signals by solid-state 17O NMR even at 35.2 T. In this work we used glucose dissolved in glycerol to simulate the slow tumbling motion of a biological macromolecule.

**Fig.1** 17O QCT NMR spectra of [3,5,6-17O3]-D-glucose dissolved in glycerol. The spectra were obtained at 270 K with 2048 and 20480 scans at 35.2 and 18.8 T, respectively.

**Conclusions**

Our results showed the significant potential of 17O QCT NMR at 35.2 T for studying biological macromolecules. The resolution improvement, coupled with the sensitivity gain associated the high magnetic field, opens the door for probing selectively 17O-labeled proteins.

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**References**

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