**13C{17O} D-HMQC Experiment**

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**Introduction**

As part of our continuing effort to develop 17O NMR into a new probe for studying biological macromolecules such as proteins, we have made progress in synthesizing amino acid type 17O-labeled proteins. In this case, a protein molecule can be synthesized where a specific type of amino acid residue is 17O-labeled. However, one of the potential problems in solid-sate 17O NMR studies of such amino acid type 17O-labeled proteins is signal assignment. That is, how can one assign the observed 17O NMR signals to the 17O-labeled amino acid residues? One possible solution is to utilize the 13C{17O} D-based HMQC experiment [1,2] for residue specific 13C=17O-doubly labeled proteins. In general, 13C NMR signals can be readily assigned from well established procedures using uniformly 13C,15N labeled protein. Then the 17O NMR signals can be assigned through their correlation to 13C. In this work, we set out to test and optimize the 13C{17O} D-based HMQC experiment with a 13C,17O doubly labeled compound, [13C,17O]Gly/GlyHCl co-crystal.

**Experimental**

All 13C{17O} HMQC experiments were performed in a midbore 18.8 T magnet at the NHMFL with a Bruker Avance III HD console and a homebuilt low-E 3.2 mm MAS 1H–X–Y probe (NHMFL #53). 13C-17O dipolar recoupling was achieved under the R3 condition [3]. Sample spinning was controlled at 18 kHz.

**Results and Discussion**

**Fig.1** shows a 2D 13C{17O} D-HMQC spectrum of [13C,17O]Gly/GlyHCl co-crystal. In this sample, the level of 13C and 17O enrichment is 100% and 20%, respectively. In the Gly/GlyHCl co-crystal, one Gly molecule is in its zwitterion form and the other is in the free acid form [4]. Thus there are two types of carboxylic acid groups: C2OO– and O=C1-OH. As seen from **Fig.1**, the observed 13C{17O} cross peaks within each type of carboxylic acid group is consistent with the crystal structure. It is also noted that there exists significant line shape distortion in the indirect 17O dimension. It appears that this kind of line shape distortion is the greatest for the C=O group. It is unclear at this time whether the line shape distortion is mainly due to a particular relative orientation between the 17O quadrupole coupling tensor and the 13C-17O dipolar vector or due to the presence of large chemical shift anisotropy at the C=O group. Currently, the overall sensitivity of the 13C{17O} D-HMQC experiment is only about 2% as compared with direct 13C CP/MAS. Of course, this can be improved if a sample with higher 17O enrichment is employed. While further improvement in recoupling efficiency is desirable, our results nevertheless showed that it is indeed possible to use 13C{17O} D-HMQC to help with 17O signal assignment.



**Conclusions**

This work demonstrates the utility of 13C{17O} D-HMQC experiment in establishing heteronuclear connectivity. In addition, it is possible to combine 13C{17O} D-HMQC with DNP. This will be a promising solution for 17O signal assignment of proteins. Work along this direction is under way in our laboratories.

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**References**

**Fig.1** 2D 13C{17O} HMQC spectrum of [13C,17O]Gly/GlyHCl co-crystal at 18.8 T. The corresponding 1D 17O MAS spectrum is shown on the side projection.

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