Dimerisation of Glutathione - Solution and Solid State Structures Studied by NMR

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Glutathione (GSH), a tripeptide (Glu-Cys-Gly) is an antioxidant present in cells which helps in removing reactive oxygen species (ROS) by its ability to undergo REDOX reaction. The oxidized form is a dimer known as Glutathione disulfide (GSSG). The ratio GSH::GSSG is a measure of cell toxicity. A special feature of the GSH tripetpide is a γ- peptide linkage with the side chain carboxylic group of Glutamic acid forming a peptide linkage with the amide of the cystine residue, which in turn is attached by normal peptide linkage to Glycine residue. During oxidation, two GSH molecules come together forming a dimer linked by a disulfide bridge. There are two ways the molecule can dimerize. (i) parallel orientation, and (ii) antiparallel orientation. Earlier solution NMR report predicted the structure to be an antiparallel dimer [1]. But interestingly crystal structure which appeared later [2] indicates a parallel dimer. To reconfirm these results, we have investigated both the monomer (GSH) and the dimer (GSSG) in solution and in solid state using NMR.

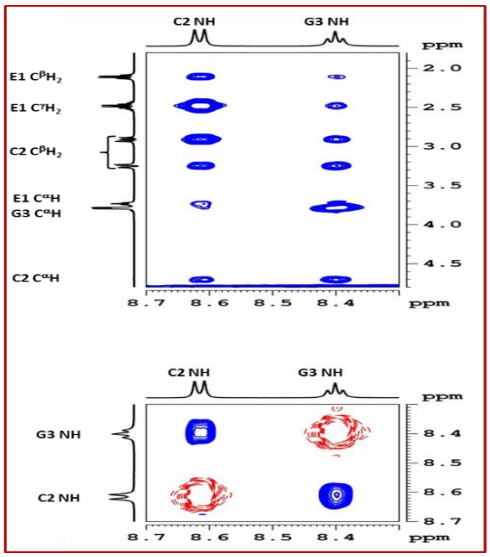

Figure 1: 700 MHz partial ROESY spectrum of GSSG. (Top) Connectivities by amide protons to various aliphatic protons. (Bottom) Amide-Amide (d_{NN}) proton connectivities.

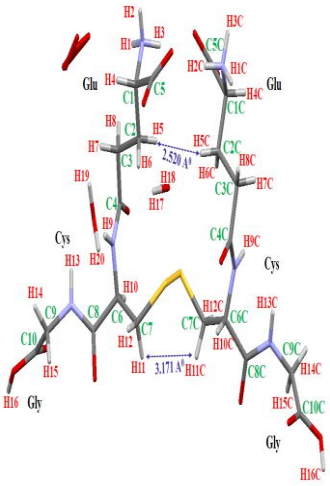

Figure 2: Structure of GSSG(SSNMR)

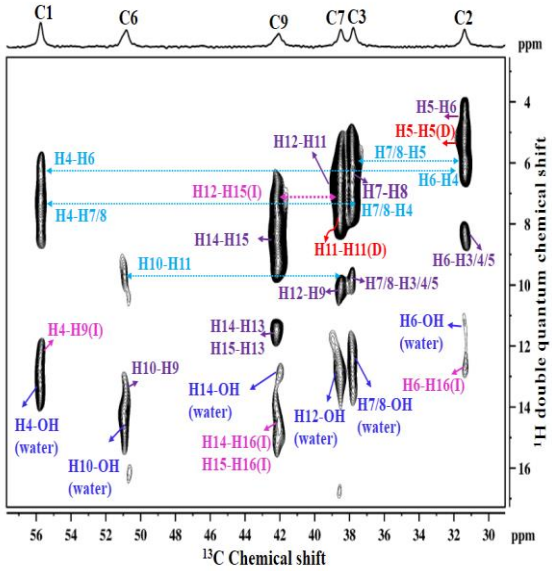

Figure 3: MAS-J-1H(DQ)-13C-HMQC spectrum of GSSG

Solution studies were carried out with 1mM water solution with pH 6.5 phosphate buffer on a 700 MHz spectrometer. 1D NMR spectra were recorded at different temperatures between 278K and 308K to delineate hydrogen boding pattern in the dimer. 2D experiments were recorded at 288K where the spectral dispersion was good. Resonance assignments were straight forward. All important NOE connectivities indicated an antiparallel dimer formation. pH titration study has also carried out to decipher whether chemical environment has effect on structure of GSSG.

In solid state NMR, ^{1}H-^{13}C heteronuclear experiments such as ^{1}H-^{13}C INEPT-HSQC [3] and MAS-J-^{1}H(DQ)-^{13}CHMQC [4] spectroscopic techniques were applied at natural abundance. Resonance assignments were independently done without bias of solution studies and were confirmed by DFT calculations. There were significant changes in the chemicals shifts of GSSG dimer between solution and solid state. Further investigations indicate the formation of a parallel dimer in the solid state. We conclude that intermolecular interactions due to crystal packing in the solid state influence the parallel dimer formation. These are absent to a great extend in solution leading to an anti-parallel arrangement

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3. B. Elena et al., Journal of the American Chemical Society, 127(49):17296-17302, 2005.
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