Toward the Solution Structure of Large Proteins and Macromolecular Complexes by Heteronuclear Pulsed-field-gradient NMR Techniques

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Purpose: Advances in NMR technology over the last 5 years, particularly the development of 3 and 4 dimensional heteronuclear NMR, have provided the tools to determine the solution conformations of medium sized biomolecules in the range of 15-25 kDa. The problem arises from the short transverse relaxation times (T2) of nuclei in larger molecules due to slower rotational correlation times and the resulting efficient dipolar relaxation between covalently bound nuclei. In this presentation, we propose that new multiple pulse techniques combined with pulsed-field-gradients and random incorporation of deuterium into protein can overcome this problem.

Materials and Methods: Triply labelled ^{2}H, ^{13}C, ^{15}N-trp repressor protein was prepared using E.coli strain CY15070 containing the overproducing plasmid pJPR2 grown in 1-2 L of minimal media. Protein-DNA complexes were made by adding the appropriate amount of synthetic operator DNA to the protein sample. All of the experiments were performed on Varian Unity600 spectrometer equipped with a triple resonance probe and an actively shielded pulsed field gradient coil. Water signal and artifacts were suppressed by a series of gradient pulses.

Results: The principle motivation for the substitution of ^{2}H for carbon-bound ^{1}H spins in ^{15}N and ^{13}C labelled proteins is the decrease in ^{13}C relaxation efficiency due to the approximately 7-fold lower gyromagnetic ratio of ^{2}H compared to ^{1}H. Pulsed-field gradients are employed in all of the experiments in order to minimize artifacts and the residual water in spectra as well as to select for the coherence transfer pathways. The data clearly indicate that spectra of high sensitivity and resolution can be obtained with ^{15}N, ^{13}C, ^{2}H labelled proteins having molecular masses on the order of 40 kDa.

Conclusion: These new triple-resonance NMR experiments combined with pulsed-field gradients techniques provide a useful approach for backbone assignment of many proteins in the 30-40 kDa molecular mass range where experiments such as 3D HNCACB on fully protonated samples often fail.