NMR determination of bakbone ^{15}N relaxation times and H-D isotope effects in a perdeuterated sample of the temperature stable protein Gb1 in the solid state.

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Structure investigations of biological solids by high- resolution magic-angle spinning (MAS) solid-state NMR spectroscopy has rapidly progressed in the last few years and resulted in complete structure elucidation of several peptides and small proteins. Successful spectral assignment and determination of structural constraints in isotopically enriched materials (mostly ^{13}C, ^{15}N) is, however, still limited by resolution and sensitivity. A gain in sensitivity in solid-state NMR (ssNMR) experiments can in principle be achieved using direct proton detection. This technique makes use of the high gyromagnetic ratio γ of protons, a property which however, leads to broad resonance lines. ^{1}H line narrowing could be achieved by isotopic spin dilution at moderate (10–20 kHz) MAS frequencies. Dilution is achieved by perdeuteration of the sample and subsequent back-exchange of deuterons by protons. Using a degree of deuteration determined by a 10:90 H_2O:D_2O mixture for recrystallization results in significant narrowing of the proton line width without loss in sensitivity. A ^{1}H line width on the order of 20–40 Hz can be achieved at moderate spinning frequencies (8–24 kHz) without application of homonuclear decoupling. The experiments are carried out using a perdeuterated, ^{15}N-enriched microcrystalline sample of the 56 residue immunoglobulin-binding domain of streptococcal protein G (GB1). This protein is an ideal model system with which to investigate protein dynamics by using NMR relaxation. It is a small, yet highly stable (*Tm* of 87 °C at pH 5.4), well-structured protein that is folded as a four-stranded β-sheet (residues 2–8, 13–20, 42–46, and 51–56), on top of which lies an α-helix running from residues 22 to 37.

In this work, we present the measurement of ^{15}N-T_1 relaxation times at different temperatures and chemical shift isotope effects in the solid state for a perdeuterated sample of the GB1 protein. Large variations of the ^{15}N relaxation time, even within the same β sheet were found. Additionally, information concerning H-D exchange rates and fractionation factors were obtained and analyzed considering hydrogen bond properties.