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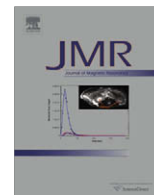

^{1}H–^{13}C hetero-nuclear dipole–dipole couplings of methyl groups in stationary and magic angle spinning solid-state NMR experiments of peptides and proteins

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ABSTRACT

^{13}C NMR of isotopically labeled methyl groups has the potential to combine spectroscopic simplicity with ease of labeling for protein NMR studies. However, in most high resolution separated local field experiments, such as polarization inversion spin exchange at the magic angle (PISEMA), that are used to measure ^{1}H–^{13}C hetero-nuclear dipolar couplings, the four-spin system of the methyl group presents complications. In this study, the properties of the ^{1}H–^{13}C hetero-nuclear dipolar interactions of ^{13}C-labeled methyl groups are revealed through solid-state NMR experiments on a range of samples, including single crystals, stationary powders, and magic angle spinning of powders, of $^{13}C_3$ labeled alanine alone and incorporated into a protein. The spectral simplifications resulting from proton detected local field (PDLF) experiments are shown to enhance resolution and simplify the interpretation of results on single crystals, magnetically aligned samples, and powders. The complementarity of stationary sample and magic angle spinning (MAS) measurements of dipolar couplings is demonstrated by applying polarization inversion spin exchange at the magic angle and magic angle spinning (PISEMAMAS) to unoriented samples.

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1. Introduction

Determining the structures and describing the dynamics of proteins requires a thorough understanding of the spectroscopic properties of the methyl group. Six of the amino acids found in proteins have methyl groups in their side chains; however, they contribute 35–40% of the residues in proteins [1]. Since valine, leucine, and isoleucine each contain two methyl groups, there are typically 60 methyl groups present in every 100 residues of a protein. The structural formulae of the methyl-containing amino acids are shown in Fig. 1. Alanine has the simplest structure; with only a single bond between the Cβ methyl carbon and the Cα-carbon, its methyl group can serve as a direct monitor of the properties of the protein backbone. Valine, leucine, and isoleucine have branched side chains, sulfur-containing methionine has a linear side chain, and threonine contains a hydroxyl group.

Methyl groups of proteins obtained by expression in bacteria can be readily labeled biosynthetically through direct incorporation of specifically labeled amino acids or through metabolic precursors labeled with ^{13}C and/or ^{2}H in various combinations [2], and this has facilitated both solution NMR and solid-state NMR studies. Many of the earlier solid-state NMR studies utilized ^{2}H NMR of deuterium-labeled methyl groups in unoriented samples to describe protein dynamics [3–6]; and some of the earliest aligned sample solid-state NMR studies of proteins involved the observation of resonances from labeled methyl sites [6].

The methyl group is unique among the side chains moieties in proteins in that it undergoes rapid reorientation about the Cβ–Cα bond axis through three-site hops among equivalent positions under physiological conditions [7], even though the activation energy for methyl-group reorientation in crystalline alanine has been shown to be relatively high at 22.6 kJ/mol [3] due to the close packing of the molecules, which is also likely to be responsible for the absence of axial symmetry in the ^{13}C chemical shift powder pattern unlike most other methyl groups in organic molecules [8–12].

We have recently described our initial solid-state NMR studies of ^{13}C-labeled methyl groups in peptides and proteins that are focused on the anisotropic ^{13}C chemical shift interaction [13]. Here we describe extensions of the spectroscopy to the ^{1}H–^{13}C dipole–dipole interactions of the methyl groups. The goal of this research is to advance the implementation of ^{1}H/^{13}C/^{15}N triple-resonance solid-state NMR experiments on stationary samples beyond that described in our earlier publications [13–24] and that of others [25]. The chemical shift and hetero-nuclear dipolar couplings associated with ^{13}C-labeled methyl groups can contribute to the determination of the complete three-dimensional structures of proteins, including all backbone and side chain sites, that are immobilized in supramolecular assemblies such as virus particles and membranes. In this article, experimental and simulated spectra from single

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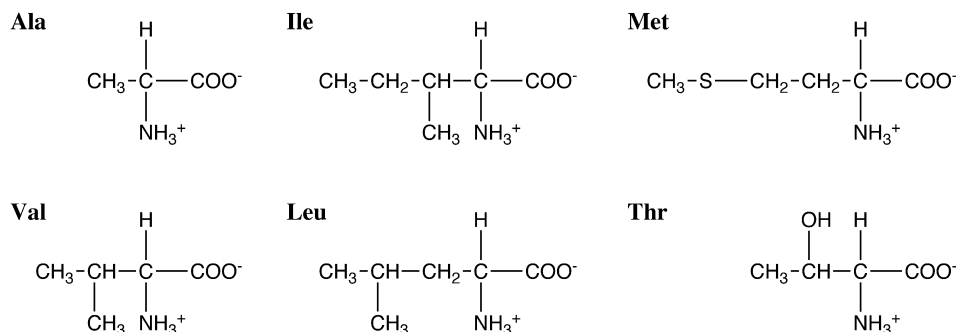

Fig. 1. Chemical structures of the methyl-containing amino acids.

crystal, uniaxially aligned, and unoriented samples in stationary and magic angle spinning (MAS) NMR experiments are integrated to provide a fuller picture of the spectroscopic manifestations of the ^{1}H–^{13}C dipolar couplings in methyl groups. By labeling only the single methyl group in each alanine residue, homonuclear ^{13}C–^{13}C dipolar couplings are minimized, enabling the focus to be on the ^{1}H–^{13}C hetero-nuclear dipolar couplings of interest. The presence of homonuclear ^{13}C/^{13}C dipolar couplings interferes with the experiments on stationary samples, and this may preclude the use of valine, leucine, and isoleucine labeled in both methyl groups in some cases. However, the use of tailored labeling, for example random fractional labeling with 25% ^{13}C [19], provides sufficient isotopic dilution so that each methyl group behaves like an isolated system.

The dipole–dipole couplings between two-spin systems are manifested as doublets in solid-state NMR spectra when they are isolated chemically or through decoupling procedures [27] from surrounding spins. The resulting high spectral resolution and direct structural information available from the splitting between the doublets have led to many studies of ^{1}H–^{15}N and ^{1}H–^{13}C spin systems. The spectral complexities associated with ^{1}H–^{13}C dipolar couplings in the three- and four-spin systems of $^{13}CH_2$ and $^{13}CH_3$ groups were observed in some of the earliest separated local field (SLF) experiments [27,28], and have been subsequently analyzed [29] and observed in studies of liquid crystals where natural abundance ^{13}C NMR experiments are feasible [30–32]. This occurs when the magnetization affected by the hetero-nuclear dipolar couplings is on the ^{13}C nucleus, in contrast to experiments where the magnetization is on the ^{1}H nuclei during the critical evolution period. The latter class of experiments is commonly referred to as proton detected local field (PDLF) spectroscopy [33], and we utilize this nomenclature for consistency, even though it is recognized that these are ^{13}C-detection experiments with the feature that the evolution of the ^{1}H–^{13}C dipolar couplings is 'carried' by the ^{1}H magnetization during the $t1$ interval where they are measured in two-dimensional spectra.

2. Results

Alanine is an effective model system for the methyl-containing amino acids in peptides and proteins. As shown in Fig. 1, with only a single methyl group, it has the simplest carbon-containing side chain of an amino acid. The experimental results presented in this section utilize samples of the amino acid alone and incorporated into a protein, and are focused on the ^{1}H–^{13}C hetero-nuclear dipole–dipole interactions of the ^{13}C-labeled methyl groups ($^{13}C_3$-) by relying on resolution of resonances based on anisotropic or isotropic chemical shift differences.

The pulse sequences diagrammed in Fig. 2 can be used to characterize the ^{13}C chemical shift and ^{1}H–^{13}C hetero-nuclear dipole–dipole interactions in $^{13}CH_3$ groups in stationary samples (Fig. 2A–D), including single crystals, polycrystalline powders, and uniaxially aligned samples, as well as those in samples undergoing magic angle spinning (Fig. 2E).

The pulse sequence in Fig. 2A is for a conventional single-contact spin-lock cross-polarization experiment [34]. SPINAL16 modulation is applied to the ^{1}H irradiation during ^{13}C data acquisition in order to have the larger bandwidth of hetero-nuclear decoupling

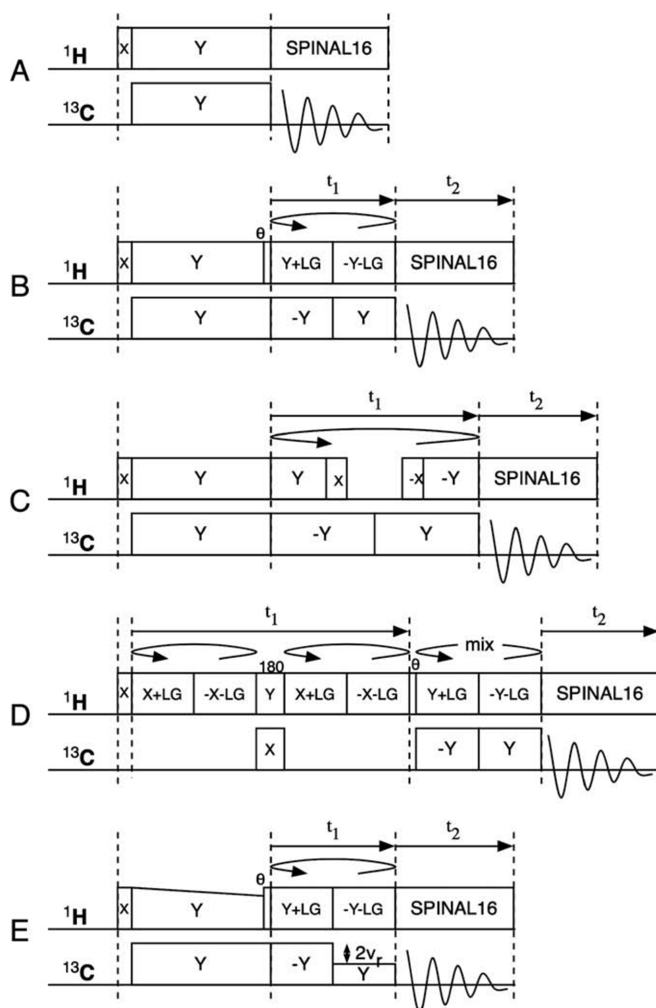

Fig. 2. Timing diagrams for the pulse sequences used to obtain ^{1}H/^{13}C solid-state NMR spectra. (A) One-dimensional single-contact spin-lock cross-polarization with SPINAL16 hetero-nuclear decoupling during data acquisition. (B) Two-dimensional PISEMA. (C) Two-dimensional SAMPI4. (D) Two-dimensional PDLF. (E) Two-dimensional PISEMAMAS.

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