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Trends

Solid-state nuclear magnetic resonance structural studies of proteins using paramagnetic probes

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ABSTRACT

Determination of three-dimensional structures of biological macromolecules by magic-angle spinning (MAS) solid-state NMR spectroscopy is hindered by the paucity of nuclear dipolar coupling-based restraints corresponding to distances exceeding 5 Å. Recent MAS NMR studies of uniformly ^{13}C, ^{15}N-enriched proteins containing paramagnetic centers have demonstrated the measurements of site-specific nuclear pseudocontact shifts and spin relaxation enhancements, which report on electron–nucleus distances up to ~20 Å. These studies pave the way for the application of such long-distance paramagnetic restraints to protein structure elucidation and analysis of protein–protein and protein–ligand interactions in the solid phase. Paramagnetic species also facilitate the rapid acquisition of high resolution and sensitivity multidimensional solid-state NMR spectra of biomacromolecules using condensed data collection schemes, and characterization of solvent-accessible surfaces of peptides and proteins. In this review we discuss some of the latest applications of magic-angle spinning NMR spectroscopy in conjunction with paramagnetic probes to the structural studies of proteins in the solid state.

Contents

1. Introduction

Over the last decade, magic-angle spinning (MAS) solid-state nuclear magnetic resonance (NMR) spectroscopy has emerged as a viable technique for probing the structure, conformational dynamics and intermolecular interactions of non-crystalline biological macromolecules [1,2] such as membrane-associated proteins [3–6], fibrillar peptide and protein amyloid aggregates [7–17], and large protein and protein–nucleic acid assemblies [18–21]. Although remarkable progress has been made in determining relatively high-resolution three-dimensional structures of small to medium size ($<$100–150 amino acids) protein molecules by solid-state NMR methods [14,22–38], such studies are generally hampered by a limited number of unambiguous long-distance ($>$5 Å) restraints that can be reliably extracted from multidimensional NMR spectra designed to measure the magnitudes of through-space magnetic dipole–dipole couplings, D_{ij}, between the protein ^{1}H, ^{13}C and ^{15}N nuclei ($D_{ij} \propto \gamma_i\gamma_j/r_{ij}^3$, where γ_i and γ_j are the

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gyromagnetic ratios of nuclear spins i and j, and r_{ij} is the internuclear distance) by using a variety of radiofrequency (RF) pulse schemes such as proton driven spin diffusion (PDSD) or dipolar assisted rotational resonance (DARR) [39–41], transferred echo double resonance (TEDOR) [42–45], CHHC, NHHC or NHHN [46,47], or proton-assisted recoupling (PAR) [33,34,48].

This scarcity of long-range distance restraints can be potentially surmounted for paramagnetic metalloprotein molecules containing high-affinity transition metal or lanthanide binding sites [49–51], or natively diamagnetic proteins intentionally modified at specific locations with covalently-bound paramagnetic tags [52–55] (see Fig. 1 for representative examples). In such paramagnetic proteins, the large electron–nucleus hyperfine couplings—stemming from the fact that the electron gyromagnetic ratio, γ_e, exceeds nuclear gyromagnetic ratios by ~2–3 orders of magnitude—manifest themselves as electron–nucleus distance-dependent pseudocontact shifts (PCSs) of the NMR frequencies and enhanced nuclear spin relaxation rates (referred to as paramagnetic relaxation enhancements or PREs). Depending on the type of paramagnetic center, one or both of these effects can be of significant magnitude for protein nuclei located up to ~20–30 Å away, which surpasses by at least three- to fourfold the range of interatomic distance restraints accessible via conventional, nuclear dipolar coupling-based solid-state NMR approaches.

The fundamental physical mechanisms underlying pseudocontact shifts and nuclear relaxation enhancements in NMR spectra of paramagnetic molecules are well understood [51,56–60], and these phenomena have been utilized since as early as the 1960s for the analysis of enzyme active sites, protein–ligand interactions, protein and nucleic acid structure and transient macromolecular binding events in solution, as thoroughly discussed in excellent reviews [61–72]. In contrast, until recently, the overwhelming majority of NMR investigations of paramagnetic biomolecules in the solid phase have centered on small metal coordination complexes [73–88], with only a handful of studies involving selectively labeled proteins [89–97]. During the past several years, however, a number of successful magic-angle spinning solid-state NMR studies of extensively ^{13}C,^{15}N-labeled protein molecules containing paramagnetic centers have been reported. These investigations include the rapid acquisition of solid-state NMR spectra of proteins with high sensitivity [98–103], the characterization of peptide insertion into lipid bilayers [104] and solvent accessibility of peptide and protein surfaces [99,105], the determination of sequential ^{13}C and ^{15}N resonance assignments [106], and site-specific pseudocontact shifts [107–109] and nuclear paramagnetic relaxation enhancements [102,110–112], as well as the initial applications of PCS [108,113] and PRE [114] restraints to protein structure elucidation. These recent studies, which are the primary focus of this review, clearly establish that unique types of structural data corresponding to length scales that are simply inaccessible by traditional solid-state NMR spectroscopy can be readily determined by using multidimensional NMR methods and applied toward the structural characterization of biological macromolecules. While still very much in their infancy, these and related MAS NMR techniques employing paramagnetic probes are likely to find widespread utility in the coming years in protein structure refinement and analysis of protein–protein and protein–ligand interactions in the solid phase.

2. Electron–nucleus interactions and their effects on NMR spectra

The Hamiltonian for a coupled electron–nucleus system in an external magnetic field, $\mathbf{B_0}$ (with magnitude B_0), can be written as [51]:

$$H = \frac{\beta_e}{\hbar}\mathbf{S}\cdot\mathbf{g}\cdot\mathbf{B_0} - \frac{g_I\beta_n}{\hbar}\mathbf{I}\cdot\mathbf{B_0} + \mathbf{I}\cdot\mathbf{A}\cdot\mathbf{S} \quad (1)$$

where $\mathbf{I}$ and $\mathbf{S}$ are spin angular momentum operators representing the nuclear and electron spins, respectively, β_e and β_n are the Bohr magneton and nuclear magneton, respectively, g_I is the nuclear g-factor specific to nucleus I (the nuclear gyromagnetic ratio, γ_I, is given by $\gamma_I = g_I\beta_n/\hbar$), $\mathbf{g}$ is the electron g-tensor which accounts for the anisotropy of the electron magnetic moment due to the orbital contribution, and $\mathbf{A}$ is the hyperfine coupling tensor that describes the interaction between the electron and nuclear spins and contains a through-space magnetic dipole–dipole contribution as well as the through-bond Fermi contact part. For simplicity, Eq. (1) assumes a $S=½$, $I=½$ system (i.e., electron zero-field splitting and nuclear quadrupole interactions can be neglected) with no chemical shielding anisotropy for the nucleus.

2.1. Paramagnetic shifts

The presence of a paramagnetic center can lead to significant frequency shifts of the nuclear resonances. One contribution to the paramagnetic shift is the Fermi contact shift, δ^{CS}, which arises from the presence of unpaired electron spin density at the nucleus. For a polycrystalline solid sample undergoing MAS this contact shift is isotropic and, in the high field approximation ($A \ll g_e\beta_e B_0$), is given by [51,60]:

$$\delta^{CS} = \frac{A}{\hbar}\frac{\overline{g}\beta_e S(S+1)}{3\gamma_I kT} \quad (2)$$

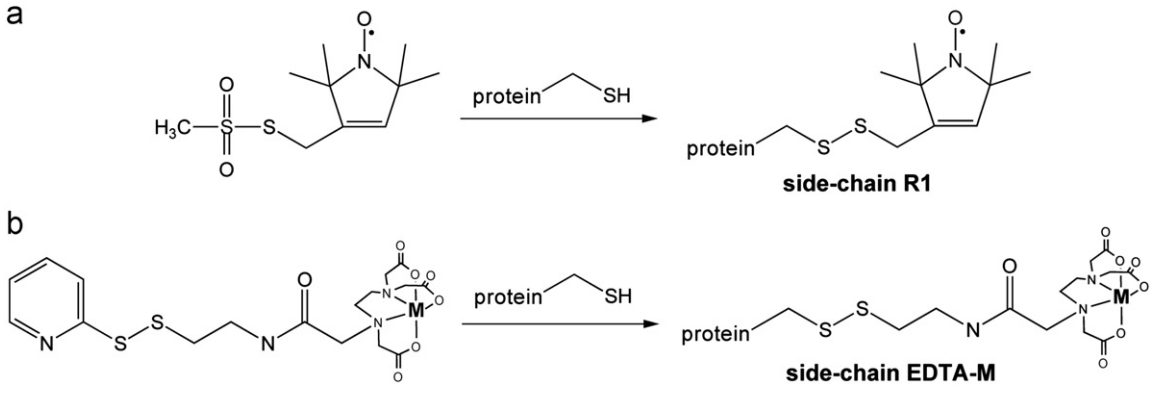

Fig. 1. Introduction of different paramagnetic tags into proteins based on cysteine site-directed mutagenesis followed by thiol-disulfide chemistry [52,53] using reagents (a) (1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl)methanethiosulfonate (MTSL) [127], and (b) *N*-[*S*-(2-pyridylthio)cysteaminyl]EDTA [128,129]. The resulting nonnative side-chains are referred to as R1 and EDTA-M, respectively. For the EDTA-M tag a variety of paramagnetic transition metal ions (M) that form tight binding chelates with EDTA can be introduced, including Cu^{2+}, Mn^{2+} and Co^{2+}. Suitable diamagnetic control samples, which are typically required for quantitative studies, include proteins tagged with side-chain R1′, containing a 1-acetyl group in place of 1-oxyl [130], and EDTA–Zn^{2+}.