Progress in Nuclear Magnetic Resonance Spectroscopy 58 (2011) 88–96

Contents lists available at ScienceDirect

Progress in Nuclear Magnetic Resonance Spectroscopy

journal homepage: www.elsevier.com/locate/pnmrs

Paramagnetic tagging for protein structure and dynamics analysis

Peter H.J. Keizers, Marcellus Ubbink *

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

ARTICLE INFO

Article history:
Received 5 July 2010
Accepted 5 August 2010
Available online 12 August 2010

Keywords:
Protein NMR spectroscopy
Paramagnetic probes
Lanthanide tags
Pseudocontact shifts
Magnetic field alignment
Paramagnetic relaxation enhancement

Contents

1. Paramagnetic effects in NMR spectroscopy . . . 88
2. Types of NMR restraints obtained from paramagnetic tags . . . 89
   2.1. Paramagnetic dipolar relaxation enhancement . . . 89
   2.2. Residual dipolar couplings . . . 89
   2.3. Pseudocontact shifts . . . 90
3. Overview of paramagnetic tags . . . 90
   3.1. Nitroxide labels . . . 90
   3.2. Metal binding peptides . . . 92
   3.3. Synthetic metal-chelating tags . . . 92
4. Applying paramagnetic tags in NMR spectroscopy . . . 93
   4.1. Protein structure refinement . . . 93
   4.2. Dynamics in proteins . . . 93
   4.3. Structures of protein complexes . . . 93
   4.4. Dynamics in protein complexes . . . 94
   4.5. Protein–ligand interactions . . . 94
5. Prospects . . . 95
   Acknowledgements . . . 95
   References . . . 95

1. Paramagnetic effects in NMR spectroscopy

Molecules containing unpaired electrons affect the magnetic properties of nuclei in their vicinity, which is observable in Nuclear Magnetic Resonance (NMR) spectra. As these effects are strongly distance dependent, the use of unpaired electrons can facilitate NMR structure determinations of (large) molecules and the complexes they form.

Abbreviations: CLaNP, caged lanthanide NMR probe; IPAP, in-phase anti-phase; PCS, pseudocontact shift; LBP, lanthanide binding peptide; NOE, nuclear Overhauser effect; PRE, paramagnetic relaxation enhancement; RDC, residual dipolar coupling; R_1^{para}, paramagnetically enhanced longitudinal relaxation rate; R_2^{para}, paramagnetically enhanced transverse relaxation rate; TROSY, transverse relaxation optimized spectroscopy.

* Corresponding author. Tel.: +31 715274628; fax: +31 715275856.
E-mail address: m.ubbink@chem.leidenuniv.nl (M. Ubbink).

doi:10.1016/j.pnmrs.2010.08.001

Already in the 1970s, Ln^{3+} ions containing unpaired electrons were employed as chemical shift and line broadening agents in NMR experiments, for instance to determine mononucleotide conformations in solution. Furthermore, it was shown that proteins like lysozyme were able to further increase the relaxation rate of the solvent water protons, which are already enhanced by Gd^{3+} [1–4]. These studies paved the way for others in which paramagnetic metals were introduced into proteins to learn about their structure. In proteins containing diamagnetic metals, such as Mg^{2+} and Ca^{2+}, the cofactor can often be exchanged for a paramagnetic one. One of the pioneering studies was reported by Lee and Sykes, later many other groups contributed to extending paramagnetic applications using Ln ions exchanged into Ca^{2+}-binding proteins [5–7].

In parallel to the use of Ln^{3+} ions, endogenous Cu^{2+} and Fe^{3+} ions, from heme and blue copper proteins, respectively, were also used to retrieve structural restraints by obtaining the paramagnetic effects from NMR experiments [8,9]. Furthermore, stable radicals were developed, in the form of small, nitroxide containing molecules readily attachable to a free thiol, allowing paramagnetic effects to be employed from NMR spectroscopy on non-metalloproteins as well [10].

In the last decade, a lot of progress has been made in the development and application of paramagnetic tags, both of the metal and stable free radical containing types. In principle, the tags can be used to retrieve paramagnetic restraints from any (macro)molecule. In this review an overview is given of the currently available paramagnetic tags, their applications are discussed and some recent examples are given. Free paramagnetic probes, those not attached to a macromolecule, have recently been reviewed elsewhere [11]. The paramagnetic tags yield information that can be used to restrain molecular structure calculations. The most commonly used types of restraints are briefly discussed first.

2. Types of NMR restraints obtained from paramagnetic tags

Paramagnetic centres are characterized by two properties that determine the type of restraints they generate. First, the electronic relaxation time, τ_S, which is the longitudinal relaxation time of the unpaired electron spin(s), is much shorter than for nuclei and ranges from microseconds down to picoseconds at ambient temperature. Centres with slow electronic relaxation (τ_S in ns–μs range) cause strong Paramagnetic Relaxation Enhancement (PRE), whereas the effect of centres with fast electronic relaxation (τ_S in ps–ns range) on nuclear relaxation is much smaller. Second, the anisotropy of the paramagnetic effect, described by the magnetic susceptibility tensor ($\Delta\chi$-tensor), causes line shifts (Pseudocontact Shifts, PCSs), as well as partial alignment of molecules in strong magnetic fields. As a rule of thumb, centres that cause extensive line broadening are not very anisotropic and thus cause negligible shifts, whereas for highly anisotropic centres, line broadening effects are limited to nuclei in a small sphere around the metal.

2.1. Paramagnetic dipolar relaxation enhancement

Unpaired electron spins may enhance relaxation rates of nearby nuclear spins because of their dipolar interaction. In solution, the fluctuating field at the nucleus that produces dipolar relaxation is caused by flipping of the electron spin due to its fast longitudinal relaxation as well as tumbling of the molecule in the magnetic field. Therefore, the relevant correlation time, τ_c, is determined by the electron relaxation time τ_S and the rotational correlation time of the molecule, τ_r, according to $\tau_c^{-1} = \tau_S^{-1} + \tau_r^{-1}$. When studying proteins ($\tau_r > 5$ ns), τ_c is often dominated by τ_S, except when the paramagnetic centre is a metal with slow electronic relaxation or a stable radical, in which case $\tau_r < \tau_S$.

Both longitudinal and transversal relaxation rates are affected by the presence of a paramagnet. The relation between longitudinal relaxation rate enhancement (R_1^{para}) and the distance from nucleus to paramagnet was described by Solomon [12]. Although R_1^{para} relaxation rates can be used to estimate distances from metals to protein nuclei, this area has not been explored much due to experimental difficulties in retrieving the paramagnetic component in the relaxation rate and from the interference of cross relaxation [13,14]. Nevertheless, using T_1 data obtained from using a freely soluble probe, in addition to a limited set of Nuclear Overhauser Effects (NOEs), the structures of small and intermediate sized proteins could be obtained [15].

The relation between the paramagnetically enhanced transverse nuclear relaxation rate, R_2^{para}, and the electron-to-nucleus distance (r_{IM}), is given by

$$R_2^{para} = \frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r_{IM}^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2}\right), \quad (1)$$

where g_e is the electronic g-factor, μ_B is the Bohr magneton, S is the total electron spin quantum number, and μ_0 is the permeability of free space. The dependence of both R_1^{para} and R_2^{para} on the squared nuclear gyromagnetic ratio (γ_I) implies that ^1H is by far the most sensitive to PRE of the common nuclei. The effects on ^{13}C and ^{15}N are 16 and 100 times less, respectively. PREs fall off with the sixth power of the distance, yielding a limited useful distance range. On the other hand, they offer the possibility to study minor conformational species of molecules in solution, as is illustrated later. Note that in (1), τ_c is used, for which an accurate value is not easily determined. Fortunately, an accurate τ_c is usually not required, because of the sixth power distance dependence of R_2^{para} on the distance. If the τ_r dominates τ_c, the protein rotational correlation can be used, but this may not always be valid, for example when using a nitroxide radical tag on the protein surface, because such a tag can have mobility independent of the protein as a whole.

Other types of relaxation-based restraints can be derived from Curie relaxation and cross-correlated relaxation mechanisms [16–18]. Up till now, paramagnetic tagging of proteins to obtain these types of restraints is a relatively unexplored area.

2.2. Residual dipolar couplings

Residual dipolar couplings (D^{res}s or RDCs) are mostly measured by using external alignment media, but with the strong and rigid lanthanide tags (Section 3), obtaining RDCs of sufficient size at 14.1 T (600 MHz ^1H frequency) is readily possible due to the partial alignment of the tag in the magnetic field. Paramagnetic alignment offers a good alternative to external alignment and has a clear advantage when studying dynamics between domains or in a protein complex, because the alignment of the observed domain can be directly related to that of the other, tagged domain, rather than indirectly via an external medium [20,21].

The RDCs are apparent as changes in the peak separations of the multiplet components in Heteronuclear Single-Quantum Correlation (HSQC)-like spectra that are acquired without decoupling, as the RDC adds to the J-coupling (Fig. 1). RDCs can be measured using the in-phase anti-phase (IPAP) pulse sequence, by acquiring J-modulated spectra, or by the recently described method by the group of Zuiderweg [22–25]. The RDC (D^{res}) depends on the angular orientation of the internuclear vector relative to the magnetic susceptibility tensor ($\Delta\chi$-tensor) of the paramagnetic centre, according to

$$D^{res} = -\frac{B_0^2}{15k_BT}\frac{\gamma_I\gamma_J h}{16\pi^3 r_{IJ}^3}\left(\Delta\chi_{ax}(3\cos^2\theta - 1) + \frac{3}{2}\Delta\chi_{rh}\sin^2\theta\cos 2\varphi\right) \quad (2)$$