Applications of paramagnetic NMR spectroscopy for protein research

Lescanne, M.

Citation

Lescanne, M. (2018, May 29). *Applications of paramagnetic NMR spectroscopy for protein research*. Retrieved from https://hdl.handle.net/1887/62455

Author: Lescanne, Mathilde
Title: Applications of paramagnetic NMR spectroscopy for protein research
Date: 2018-05-29

Chapter 7

General Discussion and Outlook

Since NMR was discovered in 1946[201,202], NMR has continuously developed to become an interdisciplinary technique used in chemistry[203-205], bio-chemistry[206-208], pharmaceutics[209,210], biology and medicine with the MRI. The use of NMR in biochemistry and particularly for protein studies became possible thanks to the constant technical progress, namely higher magnetic fields to gain in sensitivity and resolution and cryo-probes that yield 4-6 fold better signal-to-noise ratios[211,212]. From a theoretical point a of view, optimized NMR[24] pulse sequence were developed to increase sensitivity[213] as well as resolution[214]. From the wet lab point of view, new sample preparation protocols, such as perdeuteration[215,216] and other isotopic labeling[117], ensured the possibility to study bigger and bigger proteins, by preventing undesired relaxation phenomena and incrementing the number of dimensions for less crowding. Development of non-linear sampling coupled with appropriate processing[217,218] now allows the acquisition of spectra up to 5D[219] in a limited amount of time[220].

Paramagnetic NMR was part of the progress to develop liquid-state NMR further to tackle even more challenging proteins, such as large protein complexes and dynamic proteins. Originally observed in metalloproteins[221,222], paramagnetic effects were initially treated as drawbacks, mainly because paramagnetic relaxation enhancement (PRE) hampered the quality of NMR spectra. In 1994, the first liquid-state structure of a paramagnetic protein was refined by Banci et al. using NOE[223]. Since then, paramagnetic effects were used as structural restraints for protein structure determination[89,224,225], as well as dynamics[226,227] and assignment[41-43].

PARAssign applications

Several methods have been developed to assign protein spectra using different strategies and research into procedures to expand the assignment toolbox still continues to offer the NMR-user a wide choice of techniques to apply to the system of interest[228,229]. In this thesis, the possibility to assign methyl spectra using a crystal structure and PCS measured from sensitive 2D HSQC spectra was demonstrated. Assignment of spectra from simple and senstive HSQC-like spectra is of major interest for proteins suffering from low solubility or stability. It was found that the use of paramagnetic centers generating parallel susceptibility tensors must be avoided for PARAssign assignment. This alignment configuration led to redundant PCS datasets, which do not provide extra information, **chapter 2**. Consequently, the use of the same tag chelated with a different lanthanoid is predicted not to be useful for PARAssign. A simple change of the lanthanoid in the same tag would not be helpful because the $\Delta\chi$ tensor orientation is expected to be the same and the magnitude only scaled up or down, depending on the strength of the lanthanoid. However, the same mutated site could be used to work with a different tag, generating a $\Delta\chi$ tensor with different orientations. For example, it has been shown that CLaNP-5 and CLaNP-7 give sufficiently different $\Delta\chi$ tensor orientations to yield independent data sets[189].

For full assignment, the completeness of datasets remains an issue, as discussed in **chapter 2**, affecting the number of highly reliable assignments. However, despite a reduction of the fraction of assigned nuclei, those classified as highly reliable are all correct, which accounts for the robustness of the approach. In case of incomplete datasets, combining PCS with other data from other sources to identify missing assignments would be advantageous. PARAssign provides small sets of reliable suggestions for assignments, which could readily be checked by mutagenesis.

PARAssign could be used with PCS measured from TROSY spectra[230]. However, it is important to realize that an amide peak in the TROSY spectrum is shifted by $(J_{HN}+RDC)/2$ compared to the HSQC resonance in the spectrum of paramagnetic proteins because of the partial alignment caused by the lanthanoid. In the spectrum of the diamagnetic protein, the peak is shifted by only $(J_{HN})/2$. As a consequence, the difference of the proton chemical shift between paramagnetic and diamagnetic protein spectra is (PCS+RDC/2). In order to measure PCS accurately, a measure of the RDC with an IPAP experiment would be necessary. However, a RDC of 10 Hz would lead to an effect on the PCS of 0.012 ppm on the proton dimension at a field of 20T (850 MHz). These effects could be neglected without affecting the quality of the assignment.

In methyl-TROSY, the selected long-lived signal derives from a component that is not split by ^{1}H-^{13}C coupling. Therefore, it is possible to measure "RDC-free" PCS using methyl-TROSY[231]. Note that for heteronuclei the relative contribtution of the RDC to the observed shift is much larger, because the PCS expressed in Hz scales with the gyromagnetic ratio, whereas the RDC does not. Thus, PCS derived from hydrogens are more accurate than from heteronuclei.

An interesting extension of PARAssign would be the ability to assign nuclei in multimeric proteins, as discussed in **chapter 6** for the proteasome–derived α7α7 system. In many biological pathways[232], proteins associate to form protein complexes[233,234]. Homo-oligomerization[235] in particular concerns a large fraction of all proteins. Given that 100% tagging is difficult to achieve, especially in multimeric proteins, due to charge effects of the probe, analytical solutions to deal with multiple tagged protein species are desirable. Such analysis would need to take into account the possible species and the resulting PCS values. A nucleus in a dimer can exhibit four peaks, of untagged protein, or protein tagged at the near site, tagged at the far site or tagged at both sites, yielding a PCS that is the sum of the PCS caused by the tags at the near and the far sites. With higher order multimers, the number of possibilities grows rapidly, although many peaks will have very weak intensities.

PARAssign could also be enhanced to fit better experimental labeling strategies nowadays available. Methyl groups can be labelled stereo-specifically[34,236]. In the program, currently, all observable stereoisomers must be specified for each type residue, in the PARAssign configuration file. In the future, it would be better to indicate this property for each methyl group. In that way, two sets of PCS measured on two stereo-specifically labelled samples could be used together as an input with already the knowledge of the stereospecificities.

A derived application of PARAssign could ensue from its ability to determine $\Delta\chi$ tensors, using one or more paramagnetic centers, without known assignments. PARAssign's ability to determine the tensors using a PCS dataset generated from only one paramagnetic center was shown in **chapter 2**. Using methyl group PCS, $\Delta\chi$ tensors were refined to within 5 degrees error for beta angle, reporting on the main axis orientation, and between 15-25 degrees for alpha and gamma, reporting on the secondary axes orientations. Even with an incomplete set of PCS, 44 out of 76 PCS, the tensor could be refined. The possibility to determine the $\Delta\chi$ tensor of a single paramagnetic center, with a known crystal structure, without having to carry out the protein assignment first, would be a non-negligible gain of time. It could be of special interest in pharmaceutical field to quickly determine $\Delta\chi$ tensors with a reduced set of PCS, without prior knowledge of assignment. For determining ligand binding sites and orientations with transferred PCS, knowledge of the tensor parameters yields more accurate results[237]. This would enable the study of ligand binding with proton ligand NMR on large systems for which no backbone NMR spectra can be acquired, using methyl group labeling.

Structural restraints and dynamics observed with paramagnetic effects

PCS have been used already for structure calculation using proton amide PCS[89] but also proton methyl groups PCS[238]. They can also report on structural changes caused by ligand binding to a protein tagged with a paramagnetic center. Incremental changes in PCS observed during a titration with ligand report on the re-orientation of methyl group only or a more significant rearrangement of the backbone. Attention should be paid to possible tensor orientation changes upon ligand binding. Such an effect can be detected by tensor determination for the bound state and a comparison with the tensor found for the ligand-free state. In general, a global change of PCS all over the protein reports on a change in the $\Delta\chi$ tensor orientation. A local perturbation of the PCS reflects local structural changes, **chapter 4**. The mobility amplitude of the observed nucleus depends obviously on the observed PCS but also largely on the nucleus PCS gradient. The PCS gradient can be calculated to appreciate its influence on displacements for a given ΔPCS. The data in **chapter 4** illustrate the point that small changes in PCS can reflect large structural changes, in the case of a weak gradient and, analogously, large PCS changes may be caused by only tiny structural changes when the gradient is strong. The calculation of PCS gradients provides additional insight in the meaning of a PCS deviation in terms of structure. In the future, tensor calculation software could take such gradients into account as weights for the different input PCS when calculating the tensor based on a crystal or NMR structure.

Liquid-state NMR and X-ray crystallography are techniques of choice to study proteins and protein complexes at atomic resolution. However, both suffer from different limitations. X-ray crystallography, acknowledged to be very powerful to grasp the protein ground state, fails to capture potential secondary, lowly-populated states. Crystal packing can cause misrepresentations of the structure in solution. As for liquid-state NMR, it is limited by the protein/protein complex size, and the quality of the spectrum in case of non-stable or lowly soluble protein. Paramagnetic NMR is positioned to help filling the gap between these two techniques, being capable to probe low-populated states with PRE and determine structures from PCS and RDC. Paramagnetic NMR is nowadays an essential part of NMR, providing different kinds of effect that can be tuned easily to the protein of interest with different lanthanoids. The wide range of observable effects, reporting on distances as well as on dynamics is advantageously incorporated into more user-friendly software making paramagnetic NMR useful and attractive for non-NMR spectroscopists.