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Review Article

Nuclear Magnetic Resonance (NMR) Spectroscopy in Protein Research

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Abstract
Nuclear magnetic resonance spectroscopy is increasingly used in protein research. It can be used for protein structure determination in solution, close to the physiological environment. It can determine also the protein dynamics which is very important to understand biological phenomena. This article summarizes about the techniques and methods in NMR routinely used in protein research.

Keywords: Nuclear Overhauser effect, NMR, Protein

1.Introduction

Biomolecular NMR spectroscopy has been proved to be an effective tool in modern biological research in determining macromolecular structures in solution and the dynamic properties of their structures relevant to the biological functions[1]. Recent developments (eg. uniform or specific isotopic labeling, transverse relaxation optimization spectroscopy [2] residual dipolar coupling etc.) make NMR spectroscopy an exciting technique in characterizing the larger systems including protein-protein complexes and protein-nucleic acid complexes. It has the power to detect very weak interaction at atomic resolution required for the modern drug discovery processes [3]. This study aims at highlighting the use of NMR spectroscopy in protein research.

2. Parameters in NMR

2.1. Chemical shift

There are a number of observables in NMR that give information about molecular structure and dynamics. The most important one is the chemical shift. The static magnetic field B_0 is not equal to the applied magnetic field as electrons around the nucleus shield it from the applied field. Therefore the individual resonance frequencies are slightly different which reflects the different chemical environments. The resonance frequencies are termed as chemical shift. The unit of chemical shift is parts per million (ppm) in order to have chemical shift values independent of the static magnetic field.

2.2. J coupling

J coupling is the coupling between the two nuclear spins due to the influence of bonding electrons on the magnetic field between the two nuclei. It is mediated through chemical bonds connecting the two spins. Depending on the spin state of scalar coupled spin the energy levels of each spin is slightly altered. This gives rise to the splitting of the resonance lines. J couplings give information regarding the dihedral angles that can be estimated through the Karplus curve. Scalar couplings are also used in the multidimensional experiments to transfer the magnetization of one spin to another in order to identify the spin systems.

2.3. Nuclear Overhauser effect

NOESY (Nuclear Overhauser Effect Spectroscopy) spectra give inter-proton distance information in molecules if the protons are 5 Å or less apart in space. In order to calculate the structure of a protein many such distance information must be identified in an unambiguous fashion. The distance information is through space coupling and not through bond, because the dipolar cross relaxation depends on the distance between the interacting spins [1]. During analysis of a NOESY spectrum the integrated intensity of a given cross peak is interpreted in terms of the distance between the two protons giving rise to the peak. Accurate analysis requires the minimization of any effects that may systematically alter the intensity of peak. Choice of mixing time is particularly very important. Large molecules for example proteins generally give better NOEs at higher field. A midsize molecule (1000-1500MW range) may have NOEs that are close to zero and a ROESY (rotating frame Overhauser effect spectroscopy) may be required to see those molecules [1].

2.4. Residual dipolar couplings

Residual dipolar couplings (RDC) not only give additional structural information but are very much important for defining long range interactions for example the relative orientation of two protein domains. The rapid tumbling of diamagnetic molecules in solutions makes it impossible to measure the potential directional information contained in the dipolar couplings. In order to obtain the orientation restraints from residual dipolar coupling measurements the protein molecule is weakly aligned in slightly anisotropic solutions for example; 5% mixture of DHPC/DMPC forming micelles in aqueous solution, filamentous phages.

2.5. NMR spectroscopy for larger molecules: TROSY

NMR studies of larger size protein (more than 30 kDa) are mainly limited by the fact that they have larger correlation time or shorter transverse relaxation times. Signal decays more rapidly resulting in line broadening and poor resolution. A new NMR method called transverse relaxation optimized spectroscopy (TROSY) can overcome this line width problem [2]. This method extends the range of molecular size (up to 250 kDa) that can be studied by NMR. It has also been reported that TROSY-based triple resonance experiments are more sensitive than the conventional triple resonance experiments. However it generally requires high magnetic field strength.

3. Protein Structure determination by NMR

Structure determination of biological macromolecules such as proteins and nucleic acids is very much important for understanding their biological function. Moreover, protein function depends on structural rearrangement or requires appropriate changes in the structure. Identification of the relationship between structure, flexibility and function is important in providing insights into structural biology. X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy are the only two experimental methods for determination of high resolution structure of

biomacromolecules [4]. Structure determination by NMR is unique because it is possible to investigate the biological macromolecules in solution, the natural and close to physiological environment. Furthermore, NMR methods can investigate the dynamic features of the molecular structure. The process of protein structure determination by NMR spectroscopy involves three basic steps: first, the assignment of every resonance to a nuclear spin, second, the measurement of structural restraints and third, the calculation and subsequent refinement of structures that satisfy with the experimental restraints. Even for a small protein it is impossible to resolve all resonance lines in a one-dimensional (1D) spectrum. Assignments can be obtained using the sequential assignment approach based on homonuclear two-dimensional (2D) correlation spectroscopy (COSY) or total correlation spectroscopy (TOCSY) and 2D nuclear overhauser effect spectroscopy (NOESY). For larger molecules (>10 kDa.) this above assignment approach is not useful because of ovelaps between NMR signals complicates the assignment. Such systems require the addition of a third dimension by the replacement of the naturally abundant isotope ^{14}N with ^{15}N and ^{12}C by ^{13}C. Here assignment is based on triple resonance experiments allowing further decrease in the overlap of NMR signals. The resonance assignment is the bottleneck for the structure determination of the protein by NMR spectroscopy. Structural information in NMR can be obtained from different parameters. For example, chemical shifts can be used to obtain the restraints for backbone dihedral angles. Hydrogen bond restraints can be obtained by lyophilizing the protein and then dissolving in D_2O the rate of disappearance of amide signals are measured. However the most important class of restraints is NOE that establishes the correlations between proton close in space (5-6Å). NMR structure determination is heavily dependent on large number of NOEs to give inter-proton distance restraints. Finally the structure is calculated based on all these structural parameters based on restrained molecular dynamics / simulated annealing method [5]. The result of such calculation is the ensemble of structures consistent with the experimental NMR data.

4. Protein dynamics by NMR

In order to function, proteins need to undergo conformational change. Thus, dynamic properties on a broad range of time scales associated with a particular protein structure can provide very important information on specific structural changes or conformational energetics associated with the particular function. Recent improvements in the NMR experimental methods make it really a very powerful tool to monitor the dynamic behavior of the protein.

Heteronuclear relaxation experiments of isotopes ^{15}N and / or ^{13}C labeled biomolecules have become widely accepted tools for the characterization of protein dynamics. Measurement of exchange rate between the amide protons in protein and the bulk solvent can give important insight about the global stability as well as the local structural fluctuations. Slow exchange lifetimes (form minutes to days) are measured by following the loss of amide proton signal intensity of protein dissolved in D_2O.

5. Conclusion

Protein-protein interactions play an important role in many cellular events. Often such interactions are so weak and transient that it is very impossible to detect the interaction [6]. Recent methodological developments as well as the advent of high field NMR spectrometers and cryogenic probes make NMR spectroscopy an unique tool in detecting weak protein interactions in atomic details and in physiological conditions. It is also extensively applied for studying the interactions of partially or fully unfolded proteins.

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