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The study of intramolecular dynamics by nuclear magnetic resonance

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Abstract: The possibilities of studying intramolecular dynamics by nuclear magnetic resonance are discussed. It is shown that processes on a very wide time scale, ranging from picoseconds to seconds and more, can be explored by NMR. The available techniques, their potential and their limitations are demonstrated by an application to the cyclic decapeptide antamanide. By rotating frame relaxation measurements, a slow torsional mode of the peptide backbone is investigated. The proline puckering motion can be studied by J–coupling and laboratory–frame relaxation measurements. Based on combined relaxation measurements the dynamics of the phenylalanine side chains with two motional degrees of freedom are characterized. The experimental results are compared with molecular dynamics simulation calculations.

Introduction

Nuclear Magnetic Resonance (NMR) has become a very powerful and informative technique in a large variety of disciplines of natural science, reaching from solid state physics to mineralogy, all brands of chemistry, biochemistry, biology, and medicine (1–3). NMR takes advantage of the presence of nuclear magnetic moments that interact very weakly with the surroundings but are nevertheless very sensitive to the local environment. This distinguishes NMR from other structure–analytical techniques that explore more global properties, such as ultraviolet spectroscopy observing the electronic excitement of entire molecules, or vibrational spectroscopy measuring the normal modes of molecules. NMR can indeed, with its numerous "spies", obtain very detailed, highly localized information on molecules and materials. On the other hand, the shortsightedness of NMR is of disadvantage when long–range order must be studied. Fore these investigations, scattering techniques, such as x–ray, neutron and light scattering, are predestinate.

For the determination of the three–dimensional structure of molecules in solution, there is hardly a competitive technique that would match the power of NMR. In molecular biology, NMR has become, based on the pioneering work of the research group of Professor Kurt

Wüthrich, next to x-ray crystallography, the most important structure-determination technique for medium-size proteins up to about 25 000 Dalton, for nucleic acid fragments, and for oligo-saccharides. More than hundred detailed structures have already been determined in the past 10 years. Of equal importance is the study of biomolecular interactions that leads directly to an improved understanding of biomolecular function, a subject of enormous importance at present and in the near future.

It is well known that static structure or conformation is a small, although relevant aspect of the full truth. Of at least equal importance is the dynamics of molecules. Here, NMR also offers numerous attractive possibilities of detailed studies. Processes on a very wide time scale affect the nuclear magnetic resonances and can be explored. Due to the complexity of the dynamic description of all but the very smallest molecules, a full dynamic characterization is out of question, and the discussion has to be based on simplified dynamic models. However, this is not a particular limitation of NMR but is inherent in the problem itself. Again, there is hardly a competitive technique to NMR for the detailed study of intramolecular dynamics in solution.

Equally useful information can be obtained on order and dynamics in solids. Amorphous materials are particularly difficult to analyze with other means, and NMR is one of the best tools to gain insight into local ordering phenomena in macroscopically disordered materials. In addition, also dynamic processes in solids can be very well investigated by NMR. In this sense, NMR is a very powerful complement to the diffraction techniques which are most useful for the investigation of ordered crystalline materials.

That the "short-sighted" NMR method could be useful even for the study of anatomical and functional features of the human body is rather unexpected. The crucial concept is the usage of inhomogeneous magnetic fields for the encoding of spatial information in the form of shifted resonance frequencies that are proportional to the local magnetic field strength (4). This leads to magnetic resonance imaging, or MRI, a procedure that has significantly enriched the non-invasive medical diagnosis. In addition, it is also possible to perform functional studies within a living object, in the form of in-vivo magnetic resonance spectroscopy, or MRS. These exciting applications of NMR shall not be further discussed here. The main emphasis is put on molecular science.

Determination of Molecular Structure by NMR

The determination of the three-dimensional structure of a biomolecule by NMR has become a standard procedure. Geometric information is obtained from the magnetic dipolar interaction that is strictly internuclear-distance-dependent and can be determined through the measurement of cross-relaxation rates. In addition, three-bond scalar J_{kl} spin-spin interactions are related to the dihedral bond angles of H_k-C-C-H_l or H_k-N-C-H_l fragments. Based on

these two information sources, and taking into account additional constraints given by van der Waals radii and bond-angle limitations, it is possible to determine most feasible molecular conformations. Several computer-based approaches are known to reach iteratively this goal. The most successful procedures are the distance geometry algorithm (5) and restrained molecular dynamics (6). The resulting structures are normally presented in the form of superimposed families of optimized structures (2).

A most essential and critical part of the work is the measurement of the basic data, the cross-relaxation rate constants and the J-coupling constants. It requires the usage of multidimensional spectroscopy. The very first step is the assignment of the resonance lines of a proton-resonance spectrum to the numerous, often several hundred protons in the molecule. For smaller proteins with a molecular mass up to ~15–20 kDalton, a combination of a two-dimensional correlation spectrum, called COSY, that exhibits the J-coupling connectivity, and a two-dimensional cross-relaxation spectrum, called NOESY, that presents the internuclear through-space cross-relaxation rates, is sufficient for a sequential assignment of the resonance lines. After this procedure successfully finished, the measurement and interpretation as described above can follow standard procedures.

Many additional variants for the assignment of resonance lines have been worked out. Rotating frame experiments have proved to be particularly useful. For smaller molecules, often conventional cross-relaxation in the laboratory frame is weak due to an unfortunate ratio of the molecular tumbling rate and the NMR measurement frequency. In this situation, cross-relaxation measurements by the ROESY technique in the rotating frame is helpful (7). On the other hand for larger molecules, the COSY cross peaks can become very weak due to cancellation of overlapping positive and negative multiplet components. Here proton-proton J-coupling measurements in the rotating frame by the TOCSY technique can solve the problem (8,9).

For even larger molecules with a molecular mass above 20 kDalton, proton-proton J couplings lose their importance due to very broad lines. In this situation, heteronuclear one-bond couplings are of major importance. It is for example possible to assign two protons in a H_k–N–C–H_l fragment sequentially via one-bond $J_{NH'}$ $J_{NC'}$ and J_{CH} couplings. This requires, however, ^{15}N and ^{13}C labelling of the protein, a procedure that can easily be performed, provided the protein has been cloned.

At present, it seems that the limit for a full structure determination is a molecular mass of about 30 kDalton. The major problem, again, is the increasing line width with increasing rotational correlation time.

Molecular Dynamics Studies of Peptides and Proteins

Another fundamental limitation of structure–determination procedures is the presence of intramolecular motion which renders meaningless the notion of a unique molecular conformation. The characterization of molecular dynamics becomes then essential. Often, also the biological activity of a molecule is intimately connected with its intramolecular flexibility.

Intramolecular motion can be described in terms of general measures for flexibility such as auto– and cross–correlation functions. They contain little information on specific conformations that are assumed in the course of the motional process. On the other hand, it is also possible to characterize motion by a set of discrete conformations, connected by a network of rate processes.

Sources on molecular dynamics are manifold. X–ray diffraction delivers dynamic information in the form of the Debye–Waller factors B that are measures for dynamic or static disorder. Neutron diffraction reveals motional processes in the quasielastic peak. Optical fluorescence depolarization is informative about reorientational motion of e.g. tryptophane residues. Ultrasonic absorption can measure accurately rate constants, however with little information on the type of motion.

Nuclear magnetic resonance is sensitive to motion on a very wide time scale. Very slow processes with time constants in the seconds, minutes, or hours can be monitored in real time. Slow processes in the milliseconds are conveniently visualized by 2D exchange spectroscopy (10). More rapid processes in the lower milliseconds range lead to line broadening, peak coalescence, and exchange narrowing easily visible in one–dimensional spectra (11). Processes in the microseconds range can be monitored by rotating frame relaxation measurements (12), provided the process modulates an isotropic interaction parameter, such as the chemical shift or a spin–spin coupling constant. Rapid processes in the nanoseconds range are responsible for laboratory–frame relaxation (13), and intramolecular processes can be monitored unless the molecular tumbling process fully dominates the relaxation behavior. Very rapid processes in the lower picoseconds lead to averaged parameter values which can be measured, often however without providing information on the involved time scale.

In the course of structural studies of the cyclic peptide antamanide, (–Val^{1}–Pro^{2}–Pro^{3}–Ala^{4}–Phe^{5}–Phe^{6}–Pro^{7}–Pro^{8}–Phe^{9}–Phe^{10}–), it turned out that the internuclear distances measured by cross relaxation formed an incompatible set in so far as a unique three–dimensional structure is concerned (14–16). This is often a clear indication that a molecule undergoes rapid conformational dynamics between two or several structures.

A special procedure was developed for designing dynamic multiconformational models which can explain incompatible sets of distance and angular data. The procedure, called MEDUSA (Multiconformational Evaluation of Distance information Using a Stochastically constrained minimization Algorithm), constructs a large set of conformations each of which satisfies a subset of all distance constraints while violating the rest. In a second step, exchange

systems are formed that involve two or more of these conformations which are combined to fulfill all constraints (15). This procedure obtains its sense from the observation that, due to the $1/r_{kl}^6$ dependence of the distance constraints, usually only one of the involved conformations dominates one particular constraint. It is invariably the conformation with the shortest distance r_{kl}. In the determination of the proper weight of each conformation in the dynamic equilibrium, also the angular constraints derived from the J–coupling constants are taken into account.

In this manner, an entire ensemble of feasible pair structures for antamanide was selected and rated according to the fitting error for all available constraints. It turned out that the backbone of the cyclic peptide undergoes two local motional modes that cause each reorientation of a peptide–bond plane on either side of the peptide ring. This leads to four possible backbone structures which already pairwise satisfy the experimental data (16), in **qualitative** agreement with earlier investigations (14). Whether all four or only two of the possible conformations are involved in the exchange dynamics could not yet be determined with certainty. Rotating frame relaxation measurements led to an activation energy of $\simeq$21 kJ/mol and a life time of $\simeq$20 μs at 320 K for the exchange process.

In addition to this relatively slow backbone dynamics process, there is also rapid side–chain dynamics. In particular, it has been found, based on accurate measurements of J–coupling constants, that two of the four proline rings are flipping between two ring puckering states (17). Proline residues 2 and 7 are dynamic while proline residues 3 and 8 are largely fixed in one of the two possible five–ring conformations due to strain imposed by the peptide backbone. Of major importance in this context is the Karplus relation (18) that relates the vicinal J–coupling constants to the dihedral angle. By means of laboratory–frame carbon–13 relaxation measurements it was also possible to estimate the ring puckering time constant to about 30 ps in the two dynamic rings. This implies that these processes are by six orders of magnitude faster than the backbone dynamics.

Recently, the experimental findings for the proline rings could be neatly reproduced by molecular dynamics computer simulations. Two independent investigations based on the two molecular dynamics simulation programs GROMOS (19) and CHARMM (20) produced satisfactory agreement for the conformational dynamics although there is some slight disagreement between the experimental and computed time scales. In the case of the CHARMM simulation, the experimental and computed rates are equal within a factor 3.

Further studies have been concerned also with the dynamics of the other side chains. A detailed study of the motion of the phenylalanine side chains, each with two degrees of freedom has been undertaken. This study showed that NMR can be used also to study more complicated motional processes (21). It is, however, necessary to keep the dynamic models reasonably simple to avoid ill–determined systems of equations with too many unknowns.

Conclusion

During the past two decades, NMR has revealed an enormous and seemingly unlimited potential for the investigation of structural and dynamic properties of molecules and materials. It is likely that further exciting applications of this marvelous technique will be discovered in the future. There is hardly any field of science where new applications of NMR are not worth further exploration.

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