CHAPTER 9

HIGH-RESOLUTION SOLID-STATE NMR

1. INTRODUCTION

The structure, dynamics, interaction and function of biological molecules, when present in aqueous solutions, have been discussed in previous chapters. Even though, most biochemical reactions take place in cytoplasm and are thus in a solution phase, there are several situations when studies in solid state become important. One example pointed out already, pertains to molecules embedded in biological membranes. As discussed, lipids form self assembled multi-molecular structures and are integral components of biological membranes. Molecules embedded in membranes are generally not amenable to solution studies. A number of other macromolecular assemblies have restricted translational motions. As the size of the assembly increases, the molecular motions get more restricted. The immobilisation of large molecules and molecular assemblies results in the broadening of resonance lines and the behaviour of molecules approaches that in solid state. Several biological structures such as the bones are solids, while muscles and hard tissues are closer to solid rather than to solution state.

The normal NMR spectra in solid-state have fairly broad resonances and do not provide much information. In recent years, techniques have been developed which allow high-resolution spectra to be obtained in solids and solid-like biological materials. In this context, the term solid-state NMR, need not refer to materials in the usual sense of solid-state. For NMR investigations, this term usually covers situations under which molecules do not tumble isotropically, and the molecular motions are restricted to time scales of less than one msec. Such materials include both solids and semi-solids. They give very broad spectra due to line-broadening caused by dipole-dipole interactions and chemical-shift anisotropy. The spectra are normally devoid of much information.

In this Chapter, high-resolution solid-state NMR techniques are discussed. This is followed by the application of solid-state NMR for structure determination of proteins. Applications to other biological materials have been discussed in subsequent chapters.

2. NMR HAMILTONIANS IN SOLID-STATE

In solutions, where the molecules have fast isotropic motions, the NMR Hamiltonian is considered as composed of only two terms; one representing the Zeeman term (modified by the isotropic chemical shift and represented by \( \mathcal{H}_0 \)), and the other representing the J-coupling (\( \mathcal{H}_J \)). Other interactions such as through-space dipole-dipole interactions, chemical-shift anisotropy (CSA) and the quadrupolar interaction
(for nuclear spins with $I > \frac{1}{2}$), are ignored. Due to isotropic and fast molecular motions in liquids, the time averages of such interactions are zero. Thus, for rapidly tumbling molecules in isotropic situations, such as in non-viscous solutions, one ends up with relatively simple spectra and fairly narrow lines. The orientation information contained in these interactions is no longer present. Further, rapid tumbling and translational diffusion also ensure that the molecules have identical time-averaged environment.

The information on chemical shift anisotropy and dipolar couplings can be partially retrieved using solvents with anisotropic motion, or by using very high magnetic fields, which partially orient the solute molecules, as has been discussed in Chapter 4.

2.1 NMR Hamiltonian in Solid-State

In solid-state, it is necessary to consider all the terms involving interaction of nuclear spins. Equation 9.1 represents the complete NMR Hamiltonian, which consists of the following terms:

$$
H = H_\delta + H_J + H_G + H_{het(D)} + H_{hom(D)} + H_Q + H_{e,n}
$$

The terms which are common to both solid and solution states are those representing $H_\delta$ and $H_J$. These two terms are independent of the orientation of the molecule with respect to the direction of the magnetic field. In most cases, one can neglect $J$ anisotropy since it is small. However, interactions such as the CSA $[H_\delta]$, homo- and hetero-nuclear dipolar couplings $[H_{hom(D)}$ and $H_{het(D)}]$, quadrupolar interactions $[H_G]$ and electron-nuclear interactions $H_{e,n}$ contain terms which depend on the orientation of the molecule relative to the direction of the magnetic field.

The last term in Equation 9.1, arises because of interaction between the electronic and nuclear spins. As has been pointed out, this term is dominant in the presence of a free electron spin, for example, in paramagnetic molecules. The interaction between electron and nuclear spins is similar to inter-nuclear interactions, except that the electronic magnetic moment is almost 2000 times that of nuclear moments. When present, electron-nuclear interactions may dominate over the inter-nuclear interactions. NMR applications of paramagnetic ions in metallo-enzymes have been discussed in Chapter 6. The spectral changes arising from this term will not be discussed in the present Chapter.

Figure 9.1 shows approximate relative magnitudes of various intramolecular components of nuclear spin interactions.

In isotropic solvents all terms in equation 9.1 except for $H_{e,n}$ have time dependence. The time-average of such terms is zero, except for $H_\delta$ for which it is one third of the trace of the chemical shift tensor. This is the isotropic chemical shift observed in solutions, along with the scalar coupling term $H_J$. All other interactions are averaged out in solutions but are retained in the solid-state, in semi-solids such as liquid crystals, biological membranes and systems having anisotropic motions.