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Solid-state NMR and membrane proteins

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ABSTRACT

The native environment for a membrane protein is a phospholipid bilayer. Because the protein is immobilized on NMR timescales by the interactions within a bilayer membrane, solid-state NMR methods are essential to obtain high-resolution spectra. Approaches have been developed for both unoriented and oriented samples, however, they all rest on the foundation of the most fundamental aspects of solid-state NMR, and the chemical shift and homo- and hetero-nuclear dipole–dipole interactions. Solid-state NMR has advanced sufficiently to enable the structures of membrane proteins to be determined under near-native conditions in phospholipid bilayers.

1. Introduction

Membrane proteins and solid-state NMR is an important, topical area of research. NMR is the most powerful form of spectroscopy, and solid-state NMR is particularly so because of the retention of the anisotropic character of the nuclear spin interactions in the experimental results. One-quarter of all proteins encoded in the human genome are membrane proteins, and many of them have distinctive structures and unique biological functions. Membrane proteins are generally distinguished from soluble, globular proteins by having a relatively high percentage of hydrophobic residues, and by their structures being dominated to a large extent by either α-helices or β-sheet in the two major classes of membrane proteins. However, it is their association with phospholipid bilayers that uniquely distinguishes them from soluble proteins. This not only means that they are influenced by a distinctive, asymmetric chemical environment, but also, more importantly for NMR, that it immobilizes the polypeptides on the timescale of the chemical shift and dipolar coupling spin interactions. It is the immobilization of membrane proteins by their interactions with phospholipids that intimately links membrane proteins and solid-state NMR.

In unoriented, stationary samples, the chemical shift anisotropy and heteronuclear dipole–dipole interactions yield powder pattern resonances with frequency breadths of 10^4–10^5 Hz, which is the critical factor for characterizing the dynamics of membrane proteins and defining the spectroscopic requirements for obtaining high-resolution spectra. Significantly, in modern NMR experiments the radiofrequency irradiations have magnitudes >10^5 Hz, the rates of magic angle sample spinning are typically >10^4 Hz, and the receiver bandwidths can be >10^6 Hz. All of these factors combine to make high resolution solid-state NMR feasible, and are necessary for applications to membrane proteins in phospholipid bilayers.

Historically, high-resolution solid-state NMR spectroscopy and NMR of membrane proteins were developed in parallel, starting at essentially the same time, since the instrumentation and methods of solid-state NMR suitable for single crystals and powders of organic solids were also suitable for proteins and phospholipids in membrane bilayers. The initial papers describing the application of high resolution double resonance solid-state NMR to membranes [1,2] were published within a year of those introducing proton-enhanced nuclear induction spectroscopy. Notably, 50 years after high-resolution NMR spectroscopy of solids was first developed in the Waugh laboratory at M.I.T. [3–5]; the experiments continue to be the principal spectroscopic tools for NMR studies of membranes.

The origins of high-resolution solid-state NMR are the same as for the field of NMR spectroscopy itself. Following the original descriptions of the nuclear resonance phenomena [6,7], Pake demonstrated that both characteristic powder patterns and distinct doublets could be observed from solid samples of water bound to gypsum [8]. Subsequent studies showed the well-defined effects of motional averaging of powder patterns, which is an essential characteristic of many materials and especially membrane proteins in phospholipid bilayers. These motional averaging effects on homonuclear dipole–dipole [9] and chemical shift anisotropy

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[10] powder patterns are illustrated in Fig. 1. The shape and breadth of a powder pattern that is averaged by motion along a single axis reflects the angle between the principal axis of the spin interaction tensor and the axis of motion [11].

The Pake doublet [8] played several roles at the inception of NMR. It demonstrated experimentally the existence of the homonuclear dipole–dipole interaction between a pair of ^{1}H nuclei. It also demonstrated that the interaction was anisotropic, since an unoriented sample gave a powder pattern and a single crystal sample gave a single doublet for each unique site that varied with the orientation of the crystal with respect to the direction of the magnetic field. In 1948 the instrumentation and experimental methods were extremely limited in their capabilities. Thus, the Pake experiment depended on isotopic dilution. The reason that resolved doublets could be observed at all was because each of the water molecules was relatively isolated from others on the surface of gypsum. In addition to the other principles, this was the advent of isotopic dilution to isolate and narrow resonances, an approach that was re-introduced for proteins by Jardetzky and coworkers in 1968 [12], and is widely used at the present time in both solution NMR and solid-state NMR studies of proteins [13].

Isotopic dilution was also the key to the demonstration of the nature of the chemical shift interaction. The characteristic chemical shift anisotropy rotation patterns were observed in calcite ($CaCO_3$) [14]. Studies of dilute spin chemical shifts were transformed by the introduction of proton enhanced nuclear induction spectroscopy, which simultaneous increased the sensitivity and removed the severe broadening from bonded and surrounding ^{1}H nuclei in organic and biochemical compounds. This is illustrated in Fig. 2 where multiple, narrow resonances were obtained from a single crystal sample of an organic compound (durene) [15].

The third spin interaction that is used in solid-state NMR studies of membrane proteins is the heteronuclear dipole–dipole coupling, which is also an integral part of proton-enhanced nuclear induction spectroscopy. Because the severe broadening effects of the ^{1}H homonuclear couplings obscure the presence of the heteronuclear dipolar couplings, they had to be detected indirectly [16]. Only through experiments that are able to separate the effects of homo- and hetero- nuclear couplings is it possible to characterize them. Two of the most popular are rotational-echo double resonance NMR (REDOR) [17] and separated local field (SLF) [18,19] spectroscopy. More sophisticated versions of experiments that effectively decouple the homonuclear interactions while enabling the heteronuclear dipolar couplings to evolve are in current use, and are a major factor in providing high resolution multidimensional spectra of membrane proteins.

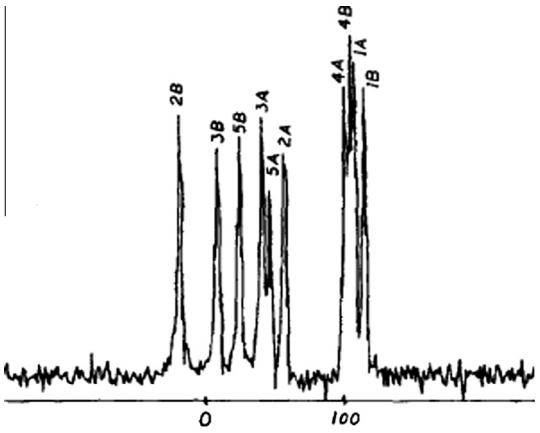

Fig. 2. High-resolution solid-state ^{13}C NMR spectra of a single crystal of durene. From Ref. [15].

2. High resolution solid-state NMR

A universal aim of NMR spectroscopy, regardless of the state of matter, is to obtain "high resolution" spectra. This means not just narrow resonance lines, as attractive as they are, but also with regard to obtaining resonances from individual sites, assigning the resonance to a particular site in the protein, and observing spectral characteristics, i.e. frequencies, line shapes, splittings, etc., that are associated with a single spin-interaction. Progress in solid-state NMR as applied to membrane proteins now fully justifies that use of the term "high resolution." Membrane proteins with as many as 350 residues have had their spectra essentially fully resolved and assigned [20].

Recent achievements are built on the fundamentals of NMR spectroscopy as summarized above. The three basic approaches to obtaining high resolution – motional averaging, spin dilution, and spin manipulation – are used in solid-state NMR studies of membrane proteins. It is worth noting that membrane proteins require the full range of solid-state NMR methods and instrumentation, and this accounts for the relatively long development period from the first double-resonance spectra of a liposome sample to the complete structures of membrane proteins that are now being determined [21].

There are multiple parts to the definition of high resolution in NMR, and one of the most important is that each resonance is associated with a single chemical site. It is essential to know which site this is. It is equally important that one spin interaction at a time is

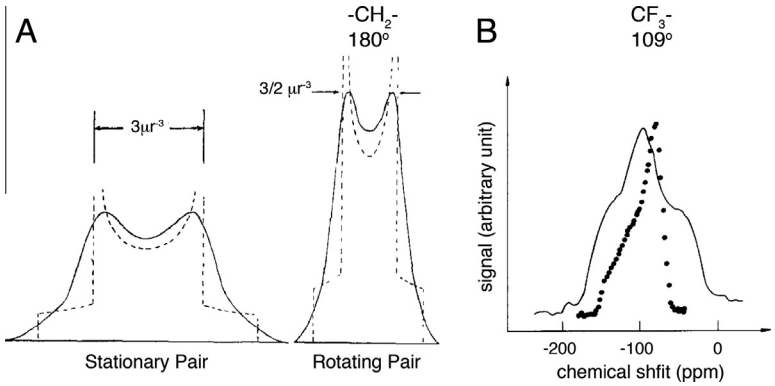

Fig. 1. The effects of rotational motion on dipole–dipole coupling and chemical shift anisotropy powder patterns. (A) Theoretical line shapes for a nuclear pair with spin ½ when stationary and when in motion about an axis perpendicular to the internuclear axis. From Ref. [9]. (B) ^{19}F powder spectra of silver trifluoroacetate at 107 K (.) and 40 K (−). The spectra are characteristic of rotating (107 K) and rigid (40 K) CF_3 groups. From Ref [10].