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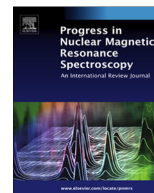

Recent advances in magic angle spinning solid state NMR of membrane proteins

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ABSTRACT

Membrane proteins mediate many critical functions in cells. Determining their three-dimensional structures in the native lipid environment has been one of the main objectives in structural biology. There are two major NMR methodologies that allow this objective to be accomplished. Oriented sample NMR, which can be applied to membrane proteins that are uniformly aligned in the magnetic field, has been successful in determining the backbone structures of a handful of membrane proteins. Owing to methodological and technological developments, Magic Angle Spinning (MAS) solid-state NMR (ssNMR) spectroscopy has emerged as another major technique for the complete characterization of the structure and dynamics of membrane proteins. First developed on peptides and small microcrystalline proteins, MAS ssNMR has recently been successfully applied to large membrane proteins. In this review we describe recent progress in MAS ssNMR methodologies, which are now available for studies of membrane protein structure determination, and outline a few examples, which highlight the broad capability of ssNMR spectroscopy.

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1. Introduction

Membrane proteins constitute approximately a third of the proteome of living cells [1]. They perform a variety of vital cellular functions including working as receptors that trigger cellular responses to external stimuli, facilitating the transport of ions, metabolites, and other solutes across lipid membranes, catalyzing reactions at the membrane interface, and mediating cell adhesion and growth. Many diseases commonly associated with mutations are linked to the malfunctions of membrane proteins, underscoring their medical importance. More than half of all market drugs are estimated to target membrane proteins.

The knowledge of membrane protein structure is one of the key factors required for detailed understanding of their function, and structure determination of membrane proteins has rapidly accelerated in recent years, largely due to the developments of new crystallization methods [2–7]. Especially impressive progress has been achieved in the characterization of G-protein coupled receptors [8–18]. Recent technological and methodological advances in solution NMR have also significantly expanded the applicability of this method to membrane proteins [19–23]. In particular, the introduction of TROSY experiments [24], isotopic labeling strategies [25–31], and advances in novel membrane mimetics, which are compatible with solution NMR [32–35], have enabled structural and dynamic analyses of membrane proteins of both β-barrel and polytopic α-helical folds [36–48].

Despite these achievements, the structural biology studies of membrane proteins significantly lags behind that of their soluble counterparts – as of April 2014 only approximately 1.4% of all protein data bank entries corresponded to the structures of membrane proteins. Furthermore, lipid mimetics, used for both crystallization and solubilization, may distort membrane protein structure [49]. Solid-state NMR (ssNMR) represents a viable and better alternative to X-ray and solution NMR methods in that it can be applied to membrane proteins reconstituted in lipid bilayers of varying lipid composition, over a wide range of pH, temperature and salinity, therefore offering the flexibility to choose optimal sample conditions which closely mimic those of cell membranes. Solid-state NMR can also be applied to the characterization of membrane proteins in native cell membranes [50–55], offering the opportunity to examine the effect of structurally and chemically heterogeneous cellular environments on protein conformation and dynamics. Not less important is that ssNMR is not principally limited by the tumbling rate of a macromolecule, and therefore structural characterization of proteins of large molecular weight is possible. The potential of ssNMR has long been recognized and discussed in the literature [56–61], however many technological and methodological challenges had to be overcome before it could be realized.

Historically, the majority of early ssNMR structures have been determined for small α-helical proteins using orientation restraints [62–69]. In this methodology, samples are uniformly aligned, either mechanically on glass plates, or using magnetically aligned bicelles, and ^{15}N–^{1}H dipolar interactions and ^{15}N chemical shift anisotropy are measured to provide restraints on both the helical tilt and local backbone structure. Such measurements generally require multiple selectively labeled samples, and are subjected to some practical limitations on the protein size. In contrast, sample preparation for Magic Angle Spinning (MAS) NMR [70,71] does not require alignment, but the experiments are more demanding, as they require relatively fast spinning for efficient averaging of anisotropic dipolar and chemical shift interactions. Recent developments in probe technology and the availability of high magnetic fields have resulted in many additional studies of large polytopic membrane proteins, and many functional and structural insights have been obtained [72–79]. In addition, it was recently demonstrated that orientation restraints can also be collected under MAS conditions using the effect of rotational alignment [80], and this approach resulted in structures for several membrane proteins, including that of G-protein coupled chemokine receptor CXCR1 [81,82].

Our main objective in this article is to highlight recent progress in MAS solid-state NMR studies of membrane proteins which have occurred over the past decade. We emphasize methods and applications to proteins with uniform and/or extensive isotopic labeling, which allows for the complete structural and dynamical characterization of a molecule. For excellent discussions of complementary Oriented Sample (OS) NMR methodology we refer readers to Refs. [83–93].

2. Preparation of isotopically labeled membrane protein samples for ssNMR studies

2.1. General requirements

Successful preparation of protein samples to a large extent defines the success of subsequent ssNMR analysis. Solid-state NMR requires large, milligram quantities of a protein, isotopically enriched with ^{13}C and ^{15}N. For the application of proton detection, membrane proteins may also need to be perdeuterated, with protons reintroduced through back-exchange. The protein has to be natively folded and reconstituted in an environment in which it is stable over the prolonged periods of time required for ssNMR experiments. As the volume of the rotors used for MAS ssNMR is limited, sensitivity considerations often dictate the use of samples with a high protein to lipid ratio. Finally, protein samples must be structurally homogeneous for optimal spectral resolution. Below we review the latest advances in membrane protein sample preparation. For a more detailed discussion of sample preparation strategies we refer readers to recently published articles and reviews [94–96].

2.2. Expression of membrane proteins

2.2.1. *Escherichia coli expression systems*

E. coli is the most widely used host for heterologous expression and isotopic labeling of proteins for ssNMR studies. Cost-effectiveness, rapid growth rates, high yields, and the availability of a large number of cloning vectors, mutant host strains, and different growth media, are the key advantages of *E. coli* expression systems. *E. coli* offers flexibility in choosing simplifying labeling strategies, which are used in ssNMR to improve spectral resolution and to

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