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Nanodiscs versus Macrodiscs for NMR of Membrane Proteins

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Supporting Information

ABSTRACT: It is challenging to find membrane mimics that stabilize the native structures, dynamics, and functions of membrane proteins. In a recent advance, nanodiscs have been shown to provide a bilayer environment compatible with solution NMR. We show that increasing the lipid to "belt" peptide ratio expands their diameter, slows their reorientation rate, and allows the protein-containing discs to be aligned in a magnetic field for oriented sample solid-state NMR. The spectroscopic properties of membrane proteins with one to seven transmembrane helices in q = 0.1 isotropic bicelles, ∼10 nm diameter isotropic nanodiscs, ∼30 nm diameter magnetically aligned macrodiscs, and q = 5 magnetically aligned bicelles are compared.

Substantial progress has been made in determining the structures of membrane proteins in recent years.[1,2] However, the major obstacles to application of NMR spectroscopy and X-ray crystallography to helical membrane proteins at the beginning of the field 35 years ago[3] are still present; these are primarily the identification and optimization of detergent/lipid environments that meet the strict sample requirements of the methods and stabilize the structures of the proteins. Micelles,[4] isotropic bicelles,[5] and other detergent/lipid assemblies are used as membrane mimics in solution NMR. Solid-state NMR is distinguished by its ability to study immobilized membrane proteins in proteoliposomes[6] and in magnetically aligned bilayers.[7] Most X-ray diffraction structures have been obtained from membrane proteins crystallized from monoolein in lipid cubic phase[8] or from detergents or bicelles.[9]

Bicelles are characterized by q, the molar ratio of the "long-chain" phospholipid (e.g., DMPC) to the "short-chain" phospholipid (e.g., DHPC) or detergent (Triton X-100 or CHAPSO). Isotropic bicelles have q <1.5, although even for small membrane proteins, q <0.5 is required for the protein to give tractable solution NMR spectra. Magnetically aligned bicelles have q >2.5, that are useful for oriented sample (OS) solid-state NMR because the integral membrane protein is immobilized and aligned along with the phospholipids in the bilayers.

Recently, protein-containing nanodiscs have been introduced as a soluble bilayer environment compatible with many biophysical experiments, including solution NMR.[10] A nanodisc is a synthetic model membrane system with a diameter of ∼10 nm, consisting of ∼150 phospholipids surrounded by a membrane scaffold protein (MSP) or amphipathic α-helical peptides derived from apolipoprotein A-1.[11] Integral membrane proteins can be inserted into the bilayer portion of the nanodiscs. Nanodiscs can accommodate a wide variety of saturated or unsaturated phospholipids. Significantly, protein-containing nanodiscs are "detergent-free", which minimizes the possibility that the membrane environment will cause the distortion or denaturation of the native protein structure.

Because nanodiscs reorient relatively rapidly in aqueous solution, they have received particular attention in the quest for conditions that yield high-resolution solution NMR spectra of membrane proteins in bilayer environments.[12−15] The principal alternatives are detergent micelles and isotropic bicelles. In solution NMR, the line widths and spectral resolution reflect the size and shape of the polypeptide, its oligomeric state, the nature of the detergent/lipid assembly, especially the q value of isotropic bicelles, and the temperature. Most efforts have been aimed at minimizing the apparent size of the protein−lipid complexes in order to decrease the rotational correlation time and obtain spectra with resonances having the narrowest line widths. In general, the line widths of resonances from membrane proteins in nanodiscs are broader than those in micelles or low-q bicelles.

The opportunities presented by increasing the diameter from ∼10 nm (nanodiscs) to ∼30 nm (macrodiscs) are shown in (Figure S1 of the Supporting Information). The discs consist of long-chain phospholipid bilayers encircled by a 14-residue peptide (Ac-DYLKAFYDKLKEAF-NH_2), a truncated analogue of class a A amphipathic α-helical peptide 18A that forms a discoidal bilayer particle when mixed with phospholipids used previously as a model for apolipoprotein A-I.[16] The diameter of the phospholipid bilayer disc can be adjusted by changing the length of the MSP[17] or the molar ratio of the phospholipids and the 14-residue peptide. The increase in diameter alters the behavior of the bilayer discs in aqueous solution. Proteins in nanodiscs undergo isotropic reorientation similar to approximately the same frequency as q = 0.5 isotropic bicelles; in contrast, protein-containing macrodiscs form magnetically alignable bilayers at temperatures above the gel-to-liquid crystalline transition temperature of the phospholipids (Figure S2), similar to the behavior of q > 2.5 bicelles.

In Figure 1, single-particle electron microscopy shows that a nanodisc has a diameter of ∼10 nm and that a typical macrodisc has a larger diameter of ∼30 nm. The difference in diameters has a dramatic effect on their ^{31}P NMR spectra. In the case of nanodiscs, the ^{31}P NMR chemical shift corresponds to the isotropic value of a phosphodiester group. In contrast,

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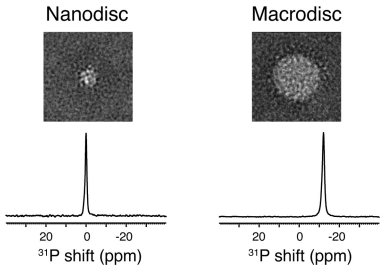

Figure 1. Representative two-dimensional class average of negatively stained protein-containing nanodiscs (left) and macrodiscs (right). Each side of a panel is 57.2 nm (top). ^{31}P NMR spectra of the phospholipids in protein-containing nanodiscs (left) and macrodiscs (right) at 40 °C. The molar ratios of DMPC to the 14-residue peptide are 1.67 for the nanodiscs and 13.3 for the macrodiscs.

macrodiscs have their ^{31}P resonance shifted by ∼12 ppm. This frequency shift is crucial evidence that the phospholipids in macrodiscs align with their normals perpendicular to the direction of the magnetic field. Moreover, it is possible to "flip" the direction of alignment of the macrodisc from perpendicular to parallel by adding lanthanide ions, as previously demonstrated for $q > 2.5$ bicelles.[18] The narrow line widths in the ^{31}P NMR spectra demonstrate that the phospholipids are arranged homogeneously, and that the macrodiscs are uniformly aligned in the magnetic field. The ^{31}P NMR data indicate that the isotropic reorientation of protein-containing nanodiscs is rapid enough for solution NMR experiments. In contrast, the macrodiscs immobilize and align the proteins along with the phospholipids for OS solid-state NMR experiments. Unoriented macrodiscs can also be used in magic angle spinning (MAS) solid-state NMR experiments.[19,20]

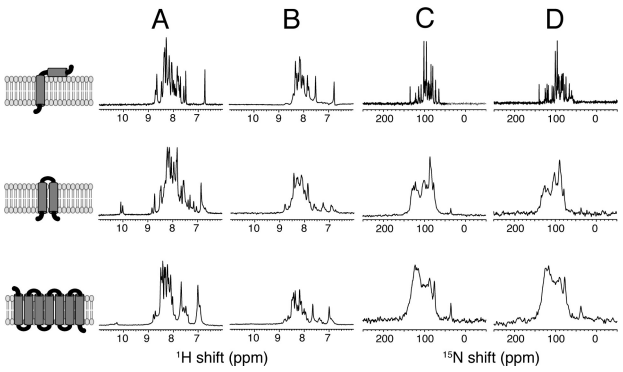

Figure 2. NMR spectra of uniformly ^{15}N-labeled membrane proteins in four different membrane-mimic environments: (A) $q = 0.1$ bicelles, (B) nanodiscs, (C) macrodiscs, and (D) $q = 5$ bicelles. From top to bottom, the left-most column contains cartoon representations of the membrane proteins with one (Pf1 coat protein), two (p7 protein), and seven (CXCR1) transmembrane helices. (A and B) ^{15}N-edited ^{1}H solution NMR spectra. (C and D) OS solid-state ^{15}N NMR spectra.

^{15}N-edited ^{1}H solution NMR spectra of the three uniformly ^{15}N-labeled membrane proteins are compared in Figure 2. Micelle samples ($q = 0$) with high detergent concentrations generally give solution NMR spectra with the best apparent resolution;[21] however, there are now multiple examples where the addition of a small amount of long-chain phospholipid improves the spectral quality. In particular, resonances missing in $q = 0$ micelle spectra can often be observed in $q = 0.1$ isotropic bicelle spectra. Both micelles and $q = 0.1$ bicelles act as effectively spherical assemblies of detergents, and the line widths of the protein resonances are similar; however, they are both problematic in terms of providing an environment conducive to the membrane protein retaining its native structure and function, especially at the high detergent concentrations and high temperatures required to obtain the best resolved spectra.[21] The membrane proteins compared in Figure 2 contain between 46 and 350 residues, and between one and seven transmembrane helices. Therefore, they represent a broad range of the helical membrane proteins found in bacteria, viruses, and humans. The top row of Figure 2 contains spectra from the membrane-bound form of Pf1 coat protein, which has 46 residues and one hydrophobic transmembrane helix.[22] The middle row contains spectra of the p7 protein from human hepatitis C virus, which has 63 residues and two transmembrane helices.[23] The bottom row contains spectra of the human chemokine receptor CXCR1, a G-protein-coupled receptor (GPCR), with 350 residues and seven transmembrane helices.[24]

For the smaller membrane proteins, signals from all amide sites are observed in the solution NMR spectra (as verified by completely assigned two-dimensional HSQC spectra). However, the resonances from the nanodisc samples are considerably broader than those from the isotropic bicelle samples. In general, protein resonances from nanodisc samples have approximately the same line widths and numbers of observable signals as in $q = 0.5$ isotropic bicelles (Figure S3). While the line widths obtained from the same protein in nanodiscs are broader than those from the same protein in $q = 0.1$ isotropic bicelles, the loss of spectral resolution may be offset by the advantages of working in a detergent-free environment. Nonetheless, it is unlikely that nanodiscs will be the optimal choice for resolution in solution NMR spectra of small membrane proteins.

The situation for CXCR1 is quite different. The only signals that can be observed in panels A and B of Figure 2 are from mobile residues at the termini of the protein.[25] Forty-four signals have been assigned to individual residues. The spectrum in Figure 2B is considerably broader and weaker. In any event, GPCRs are unlikely to yield solution NMR spectra with sufficient resolution to be useful in structural studies. We have already shown that no significant improvements result from high levels of deuteration and application of "TROSY-class" pulse sequences to CXCR1 in $q = 0.1$ isotropic bicelles.[25]

One-dimensional ^{15}N solid-state NMR spectra of the three membrane proteins are compared in macrodiscs (Figure 2C) and $q = 5$ magnetically aligned bilayers (Figure 2D). In general, the spectra appear to have slightly better resolution when the protein is immobilized and aligned in DMPC:Triton X-100 $q = 5$ bicelles[26] (Figure 2D) than in macrodiscs (Figure 2C). However, the macrodiscs have the advantage of being detergent-free; therefore, the samples have the potential to be more stable and less likely to induce distortions or denaturation of the proteins.

An entirely different use of macrodiscs in solution NMR is as an alignment medium to measure residual dipolar couplings (RDCs) of soluble, globular proteins. RDCs are an important source of structural constraints in solution NMR studies. Much like high-q bicelles,[27] macrodiscs induce weak alignment in soluble, globular proteins in aqueous solution. As an example, the measurement of ^{1}H−^{15}N RDCs for chemokine interleukin-

8 weakly aligned by the presence of macrodiscs in aqueous solution is illustrated in Figure 3. Importantly, there is no

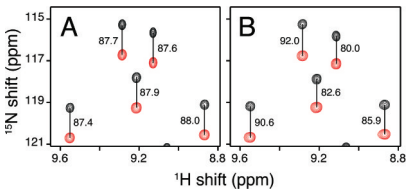

Figure 3. Representative region of $^{1}H-^{15}N$ IPAP-HSQC spectra of uniformly ^{15}N-labeled interleukin-8 at 40 °C: (A) isotropic in aqueous solution and (B) weakly aligned in aqueous solution by the addition of macrodiscs at a final DMPC concentration of 10% (w/v). The measured values of one-bond $^{1}H-^{15}N$ splitting are marked in hertz.

detectable line broadening or chemical shifts of the resonances caused by the presence of the macrodiscs in the sample.

The diameter of bilayer discs encircled by the 14-residue peptides derived from apolipoprotein A-1 can be varied by at least 3-fold by changing the lipid:peptide molar ratio. Nanodiscs and macrodiscs provide complementary lipid environments for NMR and other physical and functional studies of membrane proteins. The NMR spectra in the figures demonstrate that nanodiscs with an ~10 nm diameter and megadiscs with an ~30 nm diameter bridge between solution NMR spectroscopy and OS solid-state NMR spectroscopy. Although nanodiscs do not compete well with micelles and low-*q* isotropic bicelles for optimal resolution in solution NMR spectra, the resolution that can be attained with macrodiscs is similar to that of the best high-*q* bicelles in OS solid-state NMR spectra. Both nanodiscs and macrodiscs have the advantage of providing a detergent-free bilayer environment that reduces the likelihood of distorting or denaturing the protein structures. Finally, macrodiscs provide an alignment medium for soluble, globular proteins, allowing the measurement of RDCs. Thus, macrodiscs have the potential to play several complementary roles in NMR studies of membrane proteins.

■ ASSOCIATED CONTENT

Supporting Information

Details of experimental procedures and Figures S1–S3. This material is available free of charge via the Internet at http://pubs.acs.org.

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