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Integral Membrane Proteins in Nanodiscs Can Be Studied by Solution NMR Spectroscopy

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Investigation of large protein complexes with liquid-state NMR spectroscopy is limited by slow rotational diffusion of the particles. Increasing molecular mass translates into slower tumbling motions, causing rapid transverse relaxation of nuclear spins, broadening, and eventually disappearance of NMR signals. Transverse relaxation-optimized spectroscopy (TROSY) in combination with protein deuteration and improved hardware has pushed the virtual mass limit of solution NMR above 100 kDa for single water-soluble proteins.[1] Individual components of macromolecular complexes as big as 900 kDa have been studied with TROSY-based NMR.[2] However, integral membrane proteins (IMPs) in cellular membranes or liposomes constitute even larger particles and remain out of reach for solution NMR.

Reconstitution into "membrane mimicking" detergent micelles or isotropic lipid/detergent bicelles makes membrane proteins amenable to solution state NMR methods.[3] TROSY-based liquid-state NMR has been used to determine the structure of IMPs in detergent micelles.[4] Resonance assignment has been accomplished for large IMP/detergent assemblies.[5]

Structure and dynamics of an IMP in a detergent micelle does not necessarily reflect the native state. The micelle provides a highly dynamic environment to the IMP with detergent molecules rapidly exchanging between a monomeric state in solution and the micelle. Moreover, many detergents destabilize the native structure of proteins and compromise protein activity.[6]

Self-assembled proteolipid particles, termed nanodiscs, have been optimized for solubilization of IMPs by Sligar and colleagues.[7,8] These discoidal model membranes consist of ~150 lipid molecules arranged as a bilayer. Two copies of an apolipoprotein A-I derived, recombinant membrane scaffold protein (MSP) encircle the lipid patch and seal the hydrophobic edge of the bilayer from water. A single particle possesses a diameter of ~10 nm and a lipid-dependent thickness of ~5 nm.[8] To date, various membrane proteins have been successfully incorporated into nanodiscs in a functional form.[7,9,10] High quality solid state NMR spectra of a nanodisc-embedded IMP have been reported.[11] Nanodiscs might also prove ideal for solution NMR studies: The protein is anchored in a detergent-free lipid bilayer of adjustable composition. Nanodiscs are stable for weeks. The overall mass of the particle is below 200 kDa, i.e., within reach of TROSY-based solution NMR. Recently, a 16-mer peptide peripherally associated with a nanodisc was investigated by solution NMR.[12]

Here we present solution NMR data of an IMP reconstituted in a nanodisc demonstrating the high potential of the methodology. To our knowledge, this is the first report on this topic.

We incorporated a membrane spanning fragment of human CD4 into nanodiscs. CD4 is a type I transmembrane glycoprotein consisting of an extracellular domain of 371 amino acids, a short transmembrane region of 22 amino acids, and a cytoplasmic domain of 40 amino acids. CD4 is a coreceptor of the T cell receptor and is involved in activation of T cells.[13] Besides its various cellular activities, CD4 serves as the main receptor for human immunodeficiency virus type 1 (HIV-1).[14]

For our studies, we used CD4mut, which is a 70 amino acid residue polypeptide containing the transmembrane and cytoplasmic domains of the CD4 receptor (aa 372−433), with all five cysteine residues occurring in the native sequence being replaced by four serines and one histidine.[15] Recombinant production of uniformly ^{13}C, ^{15}N-labeled CD4mut and NMR characterization of the peptide in dodecylphosphocholine (DPC) micelles have been described before.[15,16]

Nanodiscs with and without CD4mut were assembled from the zwitterionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) and membrane scaffold protein (MSP). Experimental details can be found in the Supporting Information.

The aliphatic region of the $^{1}H-^{13}C$ HSQC spectrum of isotope-labeled CD4mut in nanodiscs containing unlabeled POPC and MSP exhibits reasonable signal dispersion in both dimensions (Figure 1A). The $^{1}H-^{13}C$ HSQC spectrum of isotope-labeled CD4mut in DPC micelles is shown in Figure 1B. The spectrum of empty nanodiscs in Figure 1C displays exclusively $^{1}H-^{13}C$ correlations of POPC arising from natural abundance ^{13}C nuclei. Resonance assignment of the lipid is presented in the Supporting Information. No cross-peaks of the unlabeled MSP were observed in agreement with the low concentration of this component (POPC to MSP molar ratio is ~60).

All POPC signals observed for empty nanodiscs (Figure 1C) are also visible in the spectrum of nanodiscs containing CD4mut (Figure 1A). The remaining cross-peaks in Figure 1A can be attributed to CD4mut. Many of the black correlation peaks in Figure 1A seem to match with nearby signals in the HSQC of CD4mut in DPC micelles (Figure 1B). Six well separated correlation peaks ($T419^{CH\beta}$, $T419^{CH3\beta}$, $F426^{CH2\beta}$, $T429^{CH\beta}$, $T429^{CH3\beta}$, $I433^{CH\beta}$) and two characteristic peak clusters ($CH2\beta$ of H399, H424, H430 and $CH3\epsilon$ of M372 and M407) have been marked with red boxes in Figure 1B. The peaks are denoted based on the published assignment.[15] Matching peaks in the HSQC of CD4mut in nanodiscs are boxed in Figure 1A. It is tempting to speculate that matching cross-peaks might reflect the same spin correlation.

All resonances marked in Figure 1B belong to amino acids in the cytoplasmic region of CD4. The ^{1}H line widths of CD4mut cross-peaks in nanodiscs are in the range from 15 to 30 Hz, very similar to the line widths of CD4mut signals observed in DPC micelles. Significantly broader lines are expected for globular

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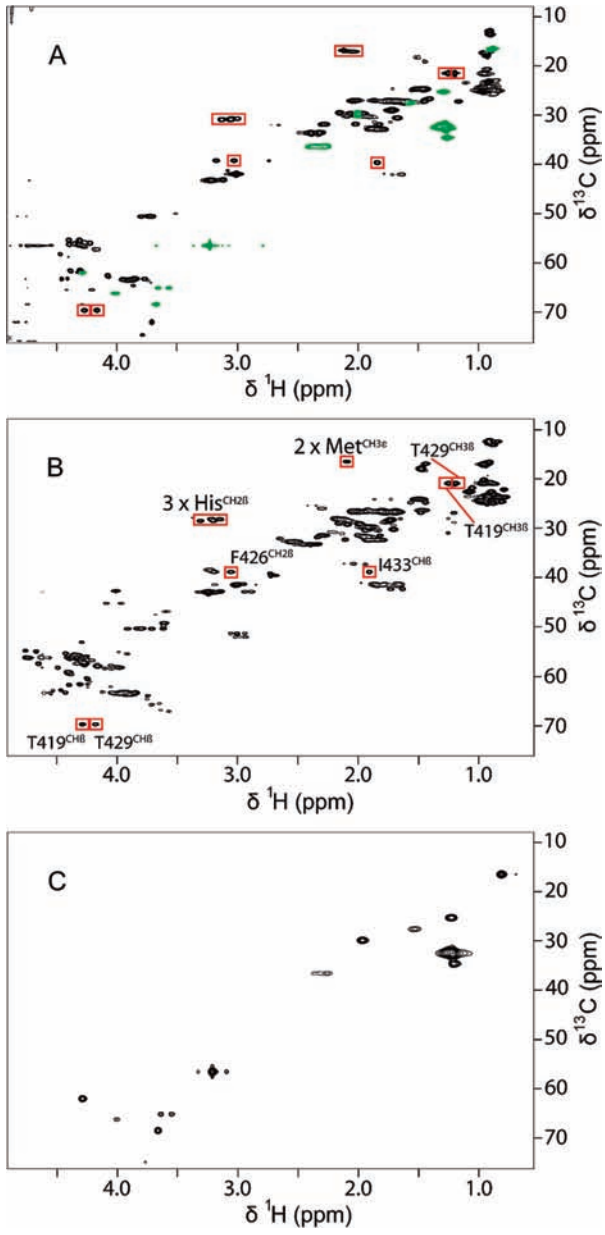

Figure 1. Aliphatic region of $^{1}H-^{13}C$ HSQC spectra. (A) CD4mut (~0.1 mM) in POPC-based nanodiscs in 10 mM sodium phosphate buffer (pH 7.4, 100 mM NaCl, 25 °C). Correlations between lipid spins are colored in green. (B) CD4mut (1 mM) in micelles (200 mM DPC-d38 in 20 mM sodium phosphate buffer in $^{2}H_2O$, pH 6.2, 150 mM NaCl, 45 °C). (C) POPC-based empty nanodiscs (~0.1 mM) in 10 mM sodium phosphate buffer (pH 7.4, 100 mM NaCl, 25 °C).

proteins of ~150 kDa that have rotational diffusion characteristics similar to those of a nanodisc. Apparently, the observed spins possess additional local mobility. Rapid axial rotation of the single transmembrane helix of CD4mut within the nanodisc will affect all CD4mut residues. A particularly high mobility is predicted for very flexible regions of the protein in the solvent exposed termini. It is conceivable that the observed protein resonances in Figure 1A arise from these mobile protein regions. $^{1}H-^{13}C$ correlations from the entire CD4mut are observed in DPC micelles (Figure 1B) resulting in more cross-peaks in comparison with Figure 1A. Small changes in CD4mut chemical shifts in nanodiscs vs micelles might reflect differences in local spin environment or in the extension of CD4mut helices.

The use of TROSY-based pulse sequences did not enhance the spectral quality in the case of the fully protonated CD4mut. However, we expect significant benefits from TROSY experiments on deuterated proteins. Nanodiscs are a very promising model system for solution NMR studies of IMPs. Nanodiscs are stable at significantly higher concentrations than the 0.1 mM used here. The membrane patch surrounding the IMP can be engineered to closely match the *in vivo* environment of the IMP. Homo- and hetero-oligomeric IMP complexes can be assembled and studied in nanodiscs under virtually native conditions.[10] The observation of NMR signals of an IMP residing in a nanodisc presents a crucial step toward solution NMR studies of IMP structure, dynamics, and molecular interactions.

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Supporting Information Available: Sample preparation, NMR data acquisition, lipid resonance assignment. This material is available free of charge via the Internet at http://pubs.acs.org.

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