Deuterium labelling applications in solid state NMR studies of membranes

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Deuterium is a non-perturbing, low gamma NMR spin probe which can be selectively incorporated chemical or biosynthetically into most biomolecules to give atomic resolution structural and dynamic information. It possesses a strong quadrupolar interaction and thus has a high of spectral anisotropy which can yield precise orientational information, as well as molecular dynamic details in the ms - μs time domain which is important in biology. We have exploited the isotope for studies of membrane structure and dynamics, either alone or by exploiting dipolar coupling to other nuclei.

We have recently focussed on the use of solid state NMR to define the structure and dynamics of binding for small, isotopically (^{2}H, ^{13}C, ^{19}F, ^{15}N) labelled ligands, prosthetic groups or solutes in their binding site of a membrane bound protein at near physiological conditions in natural membrane fragments or in reconstituted complexes [1, 2,3]. Studies of oriented membranes permit the bond vectors of labelled prosthetic groups to be determined to high resolution, as shown for retinal-d_3 in bacteriorhodopsin [4, 5, 6] and bovine rhodopsin [7] and for the agonist, acetyl choline-d_9 at its binding site in the receptor [8].

Novel magic angle spinning (MAS) NMR methods on membrane dispersions enable high resolution-like NMR spectra to be obtained for isotopically labelled ligands at their binding site in functional, membrane proteins. In particular, magic angle oriented sample spinning (MAOSS) give sensitivity enhancement of 30 – 50-fold, by reducing the wide deuterium NMR lines and focussing their intensity into spinning side bands which contain orientational and chemical shift information [9]. To yield structural information, dipolar couplings can be reintroduced into the spectrum of labelled ligands in their binding sites of membrane-bound proteins to give interatomic distances to high precision (± 0.5 Å). As examples of the use of deuterated drug analogues, the structure and binding site details for an imidazole-pyridine and ouabains, which inhibit the gastric-H^+/K^+-ATPase and Na^+/K^+-ATPase respectively, have been defined to high resolution (±0.3Å) whilst at their binding site at their membrane-bound target [10, 11, 12]. Chemical shifts can be measured directly to provide details of the chemical nature (electrostatic, hydrophobic or aromatic) binding environment for a bound ligand, as shown for acetyl choline in the acetyl choline receptor [13] and deuterium labeling provides local dynamic information for the bound agonist [14, 15] and drug partitioning [16].

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