New and Notable

Trans-Membrane Peptide and Protein Structures in Fluid Membranes via NMR

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"Transmembrane proteins serve important biological functions, yet precise information on their secondary and tertiary structure is very limited" (Altenbach et al, 1990). This remark is still valid five years after it was made.

Why has it been so much more difficult to determine structures of integral membrane than of water-soluble proteins? Paradoxically, the two most successful techniques for high-precision determination of structures of water-soluble proteins, x-ray diffraction and two-dimensional (2-D) high resolution proton (^{1}H) nuclear magnetic resonance (NMR), have been difficult to apply to integral membrane proteins but for opposing reasons involving order and disorder! Long-range translational order, in the form of good three-dimensional single crystals, is required for the accurate determination of protein structure by x-ray diffraction. Such crystals have proven to be difficult to produce until now for all but a few integral membrane proteins, although it is possible, as workers in this area frequently assert, that rapid progress is just around the corner.

By contrast, the standard method (SM) for determination of protein structure in aqueous solution by 2-D ^{1}H-NMR is based on local orientational disorder. The proteins must tumble isotropically and rapidly on the ^{1}H-NMR time scale of $\sim 10^{-5}$ s (Bloom et al., 1991). Orientational disorder associated with isotropic molecular reorientation simply does not obtain in biological membranes, which are anisotropic fluids under physiological conditions. Indeed, it has not been clear until now whether 2-D ^{1}H NMR would ever provide important structural information on integral membrane proteins in fluid membranes analogous to what is now routinely obtained on water-soluble proteins in solution. Two articles in this issue of the Biophysical Journal (Davis et al., 1995; Bouchard et al., 1995) demonstrate convincingly that it will be possible to obtain such information, at least for transmembrane peptides and small protein fragments. Although future prospects are still in doubt for obtaining structural information on more massive transmembrane proteins, this success for small peptides should stimulate new efforts to determine their structures under physiological conditions.

In the SM, the different parts of the ^{1}H-NMR high resolution spectrum are first assigned to the various amino acids making up the protein or the peptide; i.e., a correspondence is established between the components of the spectrum and the already known primary sequence. Then, geometrical relationships between different parts of the primary sequence are found using methods of 2-D NMR. The crucial role of rapid isotropic tumbling (orientational disorder) is to drastically reduce the broadening effects of the relatively large dipolar interactions on the ^{1}H-NMR spectrum. This is called "motional averaging" in the jargon of NMR. The dipolar interactions are so much larger (tens of kilohertz) than the differences in "chemical shift" (tens of hertz), which allow one to distinguish different amino acids in the high resolution ^{1}H-NMR spectrum, that a reduction of the dipolar broadening by more than a factor of 10^3 is required to make the SM work. In the case of water-soluble proteins, this requirement limits the applicability of the SM to proteins with molecular weights less than ~20,000 daltons (at the present time). Larger proteins do not tumble rapidly enough to reduce dipolar broadening sufficiently for the SM to be applied successfully.

Transmembrane peptides undergo reorientational motions in fluid membranes that are fast enough to motionally average their dipolar interactions. The motions are uniaxial rather than isotropic, however, so that the dipolar line width is only reduced by a factor of four, from 40 kHz to ~10 kHz. This motionally averaged dipolar broadening still totally masks the high resolution ^{1}H-NMR spectrum of the peptide.

In order to eliminate the dipolar broadening, Davis et al. employed a trick called Magic Angle Spinning (MAS), which is commonly used by NMR spectroscopists to reduce dipolar broadening in solids. In MAS, the spectrum is acquired, and the sample is simultaneously rotated rapidly about an axis inclined to the external magnetic field at the "magic angle," θ_m, where $\cos \theta_m = 1/\sqrt{3}$. The application of MAS to this problem by Davis et al. involved subtleties. First, it is well known that dipolar interactions between identical nuclei (^{1}H in this case) in solids always give rise to "homogeneously broadened" NMR lines, in which case the residual dipolar broadening of ~10 kHz mentioned above could not have been sufficiently reduced by MAS at attainable spinning rates. However, it was appreciated by Davis et al., after the work of the Oldfield group (1987, 1988) on lipids in membranes, that the effect of uniaxial motional averaging is not only to reduce the dipolar broadening from 40 to 10 kHz but more importantly to project the residual dipolar interactions along the axis of symmetry for the motion, i.e., along the vector **n** perpendicular to the membrane surface. The effect of this projection is to ensure that the dipolar interactions for every pair of protons on the peptide scales as $(3\cos^2\beta\text{-}1)$, where β is the angle between **n** and the external magnetic field. Thus, although the dipolar

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broadening takes the form of a continuous set of spectral frequencies, each transition within this continuous spectrum retains its "identity" (i.e., is associated with pairs of eigenfunctions that do not change) as β is changed via sample rotation, whereas the dipolar energy of the transition varies as $(3\cos^2\beta\text{-}1)$. For this highly unusual inhomogeneously broadened NMR spectrum, MAS can be used to eliminate dipolar broadening even at low spinning speeds, which are easily attainable in the laboratory.

A second astute insight by Davis et al. was that an appreciable part of the broadening still present at a spinning speed of 5 kHz, where MAS should already have been effective for such inhomogeneous broadened lines, was due to the presence of motions such as surface undulations that tilt **n** back and forth at intermediate rates so that increasing the spinning rates to 14 kHz gave narrower spectra. As pointed out by Davis et al., an improvement in the effectiveness of the MAS technique is likely to result from the elimination of the surface undulations.

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