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NMR Studies of Purple (and other) Membranes

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Abstract. We have obtained the first high-resolution solid-state proton magic-angle sample-spinning (MASS) nuclear magnetic resonance (NMR) spectra of unsonicated purple membranes, rod outer segments, and liquid crystalline lecithin bilayers; two-dimensional NMR spectra of (unsonicated) lecithin bilayers; and quantitative carbon-13 NMR spectra of selectively ^{13}C-amino acid labelled purple membranes. The carbon-13 NMR results imply rapid mobility for the C-terminus of bR. The proton results suggest increased work in this area is probably worthwhile.

We discuss in this micro-review two nuclear magnetic resonance (NMR) techniques which can (probably) be used to study photosynthetic membrane structure. The first technique, proton "magic-angle" sample-spinning (^{1}H-MASS), allows high-resolution NMR spectra to be acquired without sample sonication, while the second technique, quantitative carbon-13 NMR, gives information about the mobility of surface amino acid residues in cell membranes.

High-field ^{1}H-MASS NMR of lipids yields high resolution, multiline spectra for many liquid crystalline bilayer systems (1). In Figure 1 we show by way of example the 360 MHz ^{1}H Fourier transform NMR spectra of the smectic liquid crystal, potassium oleate (72 wt %)-D_2O, at about 25°C. The static spectrum, Figure 1A, consists of a broad, non-Lorenztian line with a width at half-height of about 2.2 kHz. The 2.7 kHz MASS spectrum, Figure 1B, shows substantial line narrowing, even at this relatively slow spinning speed. Well resolved resonances are found for CH_3, $(CH_2)_n$, and CH=CH protons. These results suggest that relatively weak intra-pair (CH_2) proton-proton dipolar coupling is the predominant source of line broadening in these systems, and ^{1}H-MASS will probably be most useful for studies of liquid crystalline phase lipids, rather than gel phase lipids, where strong intermolecular dipole-dipole interactions are expected to dominate (1).

The high sensitivity afforded by ^{1}H-MASS facilitates the application of two-dimensional (2-D) NMR techniques to unsonicated membrane systems. In Figure 2 we show a 2-D contour plot of a 500 MHz NOESY spectrum of unsonicated DMPC (50 wt %)-D_2O, with a one-dimensional spectrum along the top, for assignment purposes. Cross-peaks arise from

cross-relaxation between adjacent groups, and are indicative of close spatial proximity. We find cross-peaks between terminal methyl and hydrocarbon chain methylene protons, $^{+}NMe_3$-$(CH_2)_n$, HO^2H-$^{+}NMe_3$, HO^2H-$(CH_2)_n$, and a very weak cross-peak between the terminal methyl and $^{+}NMe_3$ protons. These results are in contrast to similar experiments performed on sonicated DPPC systems (2) where chain interdigitation and/or bending occurs.

In Figure 3A we show the 500 MHz 2.1 kHz ^1H-MASS NMR spectrum of unsonicated purple membranes, lyophilized three times from D_2O, then resuspended in D_2O. The resolution is rather low, due in part to the large residual HO^2H signal. In Figure 3B we show the 2.3 kHz ^1H-MASS NMR spectrum of unsonicated rod outer segments (ROS), lyophilized three times from of D_2O, then resuspended in 0.068 M borate buffer, pD = 7.0. Numerous well resolved resonances from the major lipid groups are observed, and the overall resolution is approximately equal to that of sonicated ROS (3).

The second technique we have employed in the study of photosynthetic membranes is quantitative ^{13}C NMR. Our goal is to get quantitative structural information on the types and numbers of amino acids in bacteriorhodopsin that are mobile, and to relate this information to the postulated folding patterns of bR.

We have biosynthetically incorporated ^{13}C labelled amino acids into bR, and have performed gas chromatography-mass spectrometry on the N-trifluoroacetyl derivatives, to determine the enrichment in ^{13}C (4). This step is critical, since we have found widely varying levels of

enrichment, even when comparing different batches of the same carbon labelled amino acid. These differences are probably due to small changes in the growth conditions.

Quantitative NMR has been performed using sucrose as an internal standard for spectrum quantification. Spin-lattice relaxation times (T_1's) were measured using the inversion recovery sequence, and all spectra were acquired at recycle time greater than 5 x T_1. Figure 4 shows the coupled 125 MHz ^{13}C NMR spectrum of $^{13}C^{o}$-leucine labelled bacteriorhodopsin, to which approximately 30 mg of sucrose was added. The resonance at about 175 ppm is from $^{13}C^{o}$-leucine residues in bR, while the peaks to the right are from the twelve (coupled) carbons of sucrose. Peak areas were measured by integration. Knowing the concentrations of sucrose and bR, along with the ^{13}C enrichment in bR, it is possible to calculate the number of mobile amino acids that are responsible for the carbonyl intensity. Seven amino acids: arg, gly, ile, leu, phe, tyr, and val, were examined in this manner. The results suggest that the C-terminus of bR is mobile (on the ^{13}C NMR time scale), in basic agreement with electron diffraction data (5) and recent fluorescence results (6).

Acknowledgment

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Figure Captions

Figure 1. 360 MHz static and MASS ^1H Fourier transform NMR spectra of potassium oleate (72 wt%)-D_2O in the liquid crystalline phase. A, static spectrum at 26°C. B, 2.7 kHz MASS NMR spectrum.

Figure 2. 500 MHz two-dimensional cross-relaxation (NOESY) contour plot of DMPC (50 wt%) in D_2O, using a mix time of 500 msec, at 26°C, and a MASS spinning speed of 3.1 kHz. Assignments of the one-dimensional spectrum are given along the top of the figure.

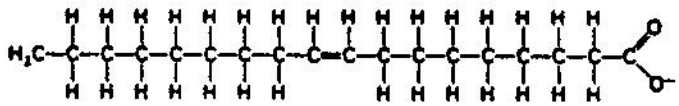
Figure 3. 500 MHz ^1H Fourier transform MASS NMR spectra of photosynthetic membranes. A, unsonicated purple membranes, lyophilized three times from D_2O, then resuspended in D_2O, at a spinning speed of 2.2 kHz. Spinning side bands from D_2O are indicated with a ★. B, unsonicated bovine rod outer segments lyophilized three times from D_2O, then resuspended in 0.068 M borate buffer, pD = 7.0, at a spinning speed of 3.6 kHz. Sidebands from D_2O are indicated with a ★, from olefenic protons with a ■, and from methylene protons with a ◆.

Figure 4. Absolute quantitation of mobile leucine in bacteriorhodopsin *via* addition of sucrose. Coupled 125 MHz solution ^{13}C Fourier transform NMR spectrum of $^{13}C^{o}$-leucine labelled bR and sucrose, recycle time 12 sec, 18 μsec 90° pulse excitation.

A

Potassium oleate - 82 wt%
D_2O - 18 wt%

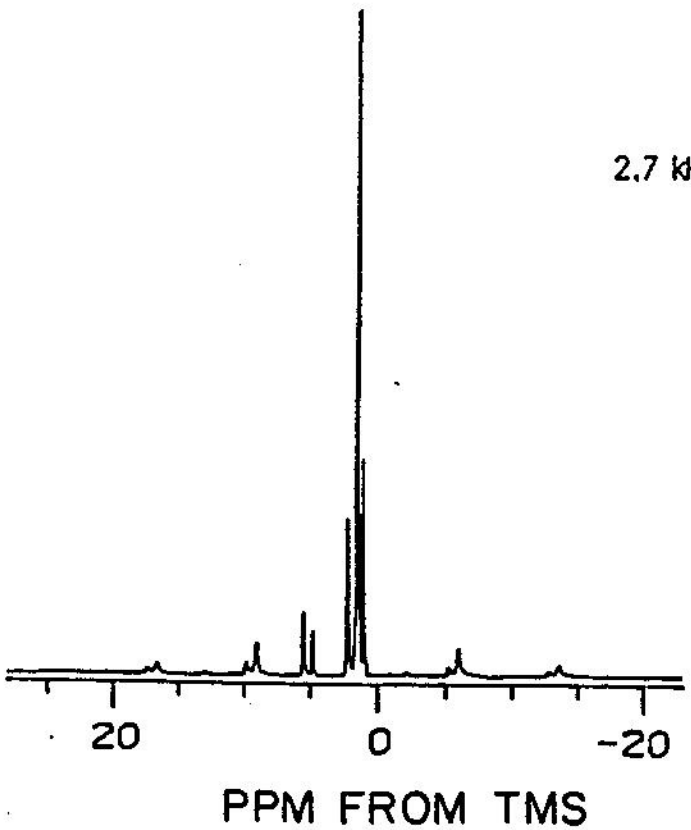
360 MHz ^1H
Static



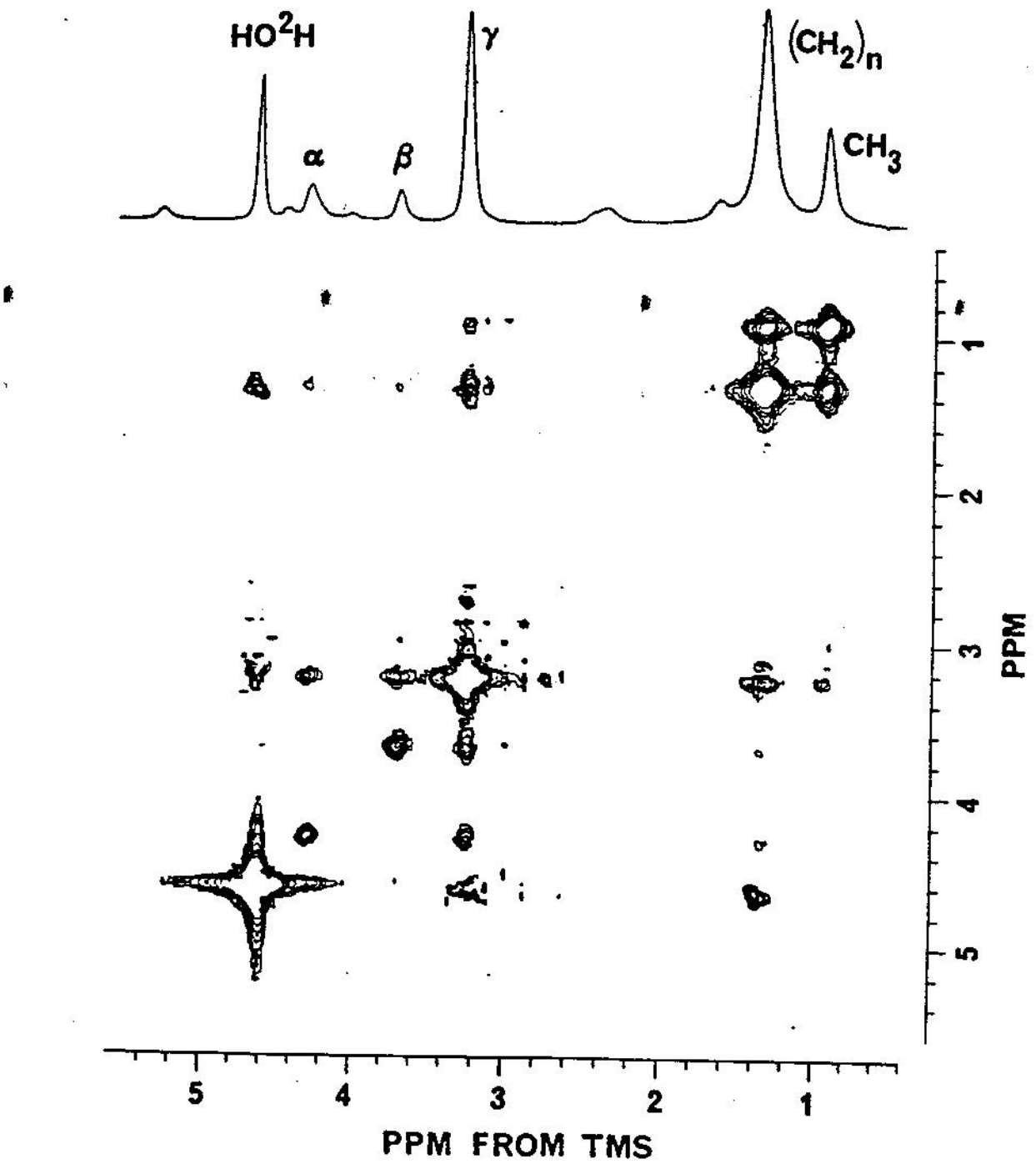
Oleate
(Ionized form of oleic acid)

B

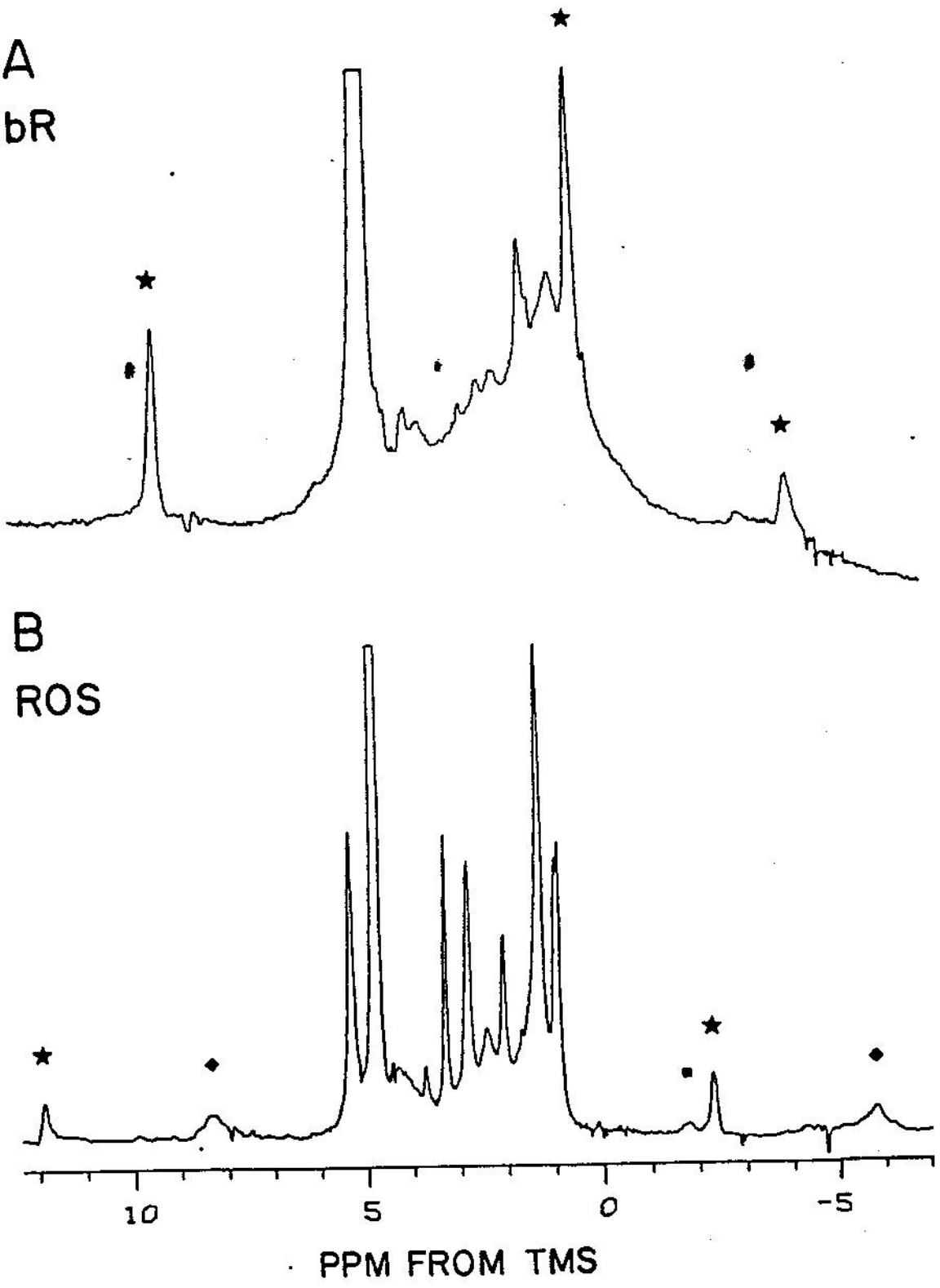
2.7 kHz MASS



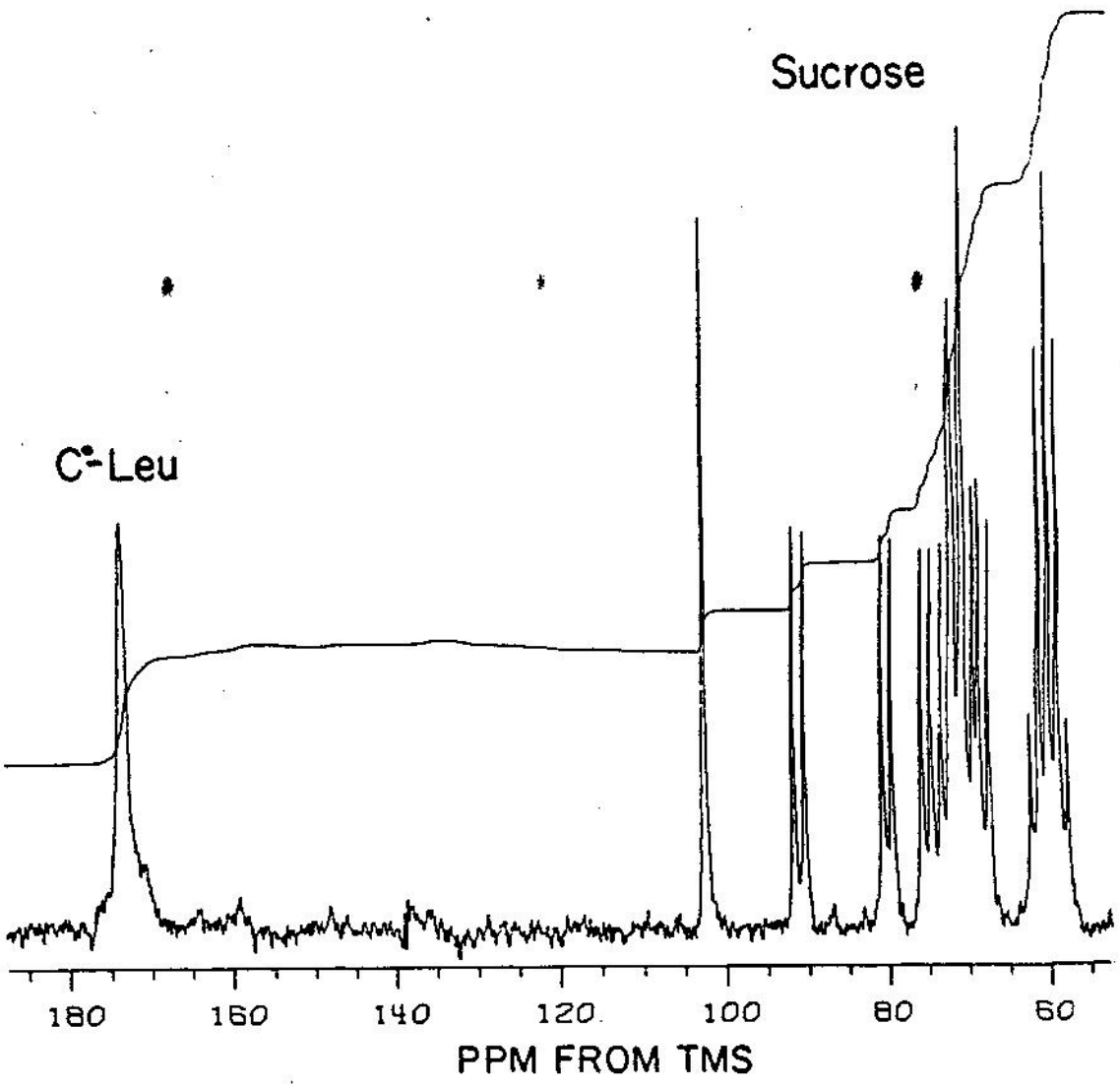
Bowers, et. al. Fig. 1



Bowers, et al., Figure 2



Bowers, et al., Figure 3



Bowers, et al., Figure 4