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A flat-coil NMR probe with hydration control of oriented phospholipid bilayer samples

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Summary

A novel flat-coil solid-state NMR probe, capable of controlling the hydration of oriented phospholipid bilayers in the course of long-term experiments, is described. Perfect hydration control for at least five days of intense radio-frequency pulsing is demonstrated using ^{31}P NMR of oriented dimyristoylphosphatidylcholine bilayers. The probe design will be of particular importance for studies of peptides and proteins oriented in lipid bilayers.

The flat-coil NMR probe design, recently described by Bechinger and Opella (1991), has shown good performance with respect to radio-frequency (rf) efficiency and sensitivity in studies of orientation, dynamics and interactions of the lipid or peptide/protein components in membrane bilayers oriented between glass plates (Bechinger et al., 1991,1992,1993). Because of the slow overall reorientation rates of such systems, these studies employ solid-state NMR techniques.

Aimed at a differentiation between trans and in-plane orientations of helical peptides in membranes, we recently conducted a series of oriented membrane studies using a home-built flat-coil NMR probe designed according to our experience in the construction of efficient high-resolution (CP/MAS) solid-state NMR probes. In the course of these studies it became evident that the hydrated lipid membrane system (layered between a stack of 15–20 glass plates) starts deteriorating after 12–24 h of NMR pulsing because of dehydration caused by rf heating. The resulting change in mobility and subsequent breakdown of sample characteristics occurs despite the fact that the stacked samples were kept in a stream of humidified air at room temperature, as recently proposed (Bechinger et al., 1993). Clearly, dehydration becomes a severe problem in studies of oriented peptides and proteins of low concentration and low inherent NMR sensitivity (e.g. ^{15}N or ^{13}C) since these may require several days of accumulation. This communication describes a flat-coil NMR probe, designed to control the hydration of membrane samples through the use of a combined sample and hydration housing with separate variable temperature controls of the two compartments. Using this equipment, control of the hydration and thereby uniform mobility and conformation of headgroups within hydrated phospholipid bilayers can be maintained for at least five days of rf pulsing.

Dehydration of oriented membrane samples in the course of an NMR experiment may introduce several experimental difficulties. First, the initial dehydration renders the sample nonuniform with respect to molecular mobility. This causes difficulties not only in studies of molecular dynamics, but also in studies of molecular structure when the information must be extracted on the basis of anisotropic interactions partially averaged by molecular motion. Second, on a longer time scale dehydration destroys the overall orientation of the phospholipid sample, implying a severe loss of information and sensitivity. Third, the conformation of the lipid headgroups within the membrane (Griffin, 1981; Ulrich et al., 1990; Bechinger and Seelig, 1991; Morrison, 1993) and the orientation of peptides and proteins dispersed in an

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oriented phospholipid bilayer (Huang and Wu, 1991; De Jongh et al., 1994) may depend on the degree of hydration of the membrane. Finally, dehydration may cause gradual detuning of the probe, thereby lowering the signal-to-noise ratio.

Various attempts to solve the problem of membrane dehydration have appeared in the literature. These include sealing the oriented sample in an air-tight sample chamber (Nicholson et al., 1987; Cornell et al., 1988) or keeping the bilayer sample in a stream of humidified air (Bechinger et al., 1993). However, to our knowledge no evidence exists that either method prevents dehydration in long-term experiments; in fact, in the present study dehydration is observed under such conditions. A different approach is to surround the sample by a solution of water in polyethylene glycol, a method claimed to be superior to hydration using water-saturated air and to ensure hydration stability over times as long as a week (Morrison, 1993). Although the latter method appears useful, it is often desirable to avoid contamination of the sample with the polymer, especially when an expensive isotope-labeled peptide or protein is incorporated into the lipid bilayer.

Figure 1 illustrates the probe (Fig. 1A) with the combined hydration/sample housing (Fig. 1B), constructed during this work for efficient control of the hydration of oriented membrane samples in solid-state NMR. A free-standing four-turn flattened coil (flattened copper wire, 0.5×1.0 mm) of inner dimensions 8 (length) $\times$ 14 (width) $\times$ 4 (height) mm ensures excellent sensitivity and rf efficiency. The coil is double-tuned (X, ^{1}H) to allow cross-polarization (CP) and/or ^{1}H dipolar decoupling (DD) in, for example, ^{31}P-^{1}H and ^{15}N-^{1}H CP/DD NMR experiments. The sample chamber of the housing (Fig. 1B, part I), produced from a single piece of Macor (Corning Glass Works, Corning, NY), fits tightly into the rf coil and to achieve the highest possible filling factor, the wall thickness of the chamber is machined to 0.3 mm. An end-cap stopper (Macor) with one O-ring (Fig. 1B, part II) ensures an air-tight seal of the sample and hydration housing. The dimensions of the complete housing are shown in Fig. 1B. A stack of about 15 thin glass plates (dimensions $10 \times 10 \times 0.1$ mm) with a layer of about 5–10 mg of the phospholipid sample between contiguous plates can be placed inside the chamber, which has its horizontal plane aligned perpendicular to the magnetic field.

To maintain a humid atmosphere within the sample chamber, a plug of wet cotton can be placed inside the reservoir of the end cap (Fig. 1B, part II). However, using this set-up, actual ^{31}P NMR experiments (not shown) for an oriented sample of dimyristoylphosphatidylcholine (DMPC) show that deterioration/dehydration is still observed after about 15 h, similar to using the air-tight housing without a wet cotton plug (vide infra). This indicates that a temperature gradient between the sample and the hydration chamber builds up due to rf heating from the efficient ^{1}H decoupling ($\gamma_H B_{1H}/2\pi = 50$–$60$ kHz) generally required for averaging the heteronuclear dipolar couplings to the abundant protons. For example, for a 1% duty cycle of the decoupler we measure a temperature of 36 °C for a lipid sample; the remaining part of the probe has a temperature of 23 °C (ambient). Air cooling (ambient temperature, 23 °C) of the complete sample and hydration housing did not result in improvements to the experimental observations. It is concluded that the temperature gradient between the two chambers prevents

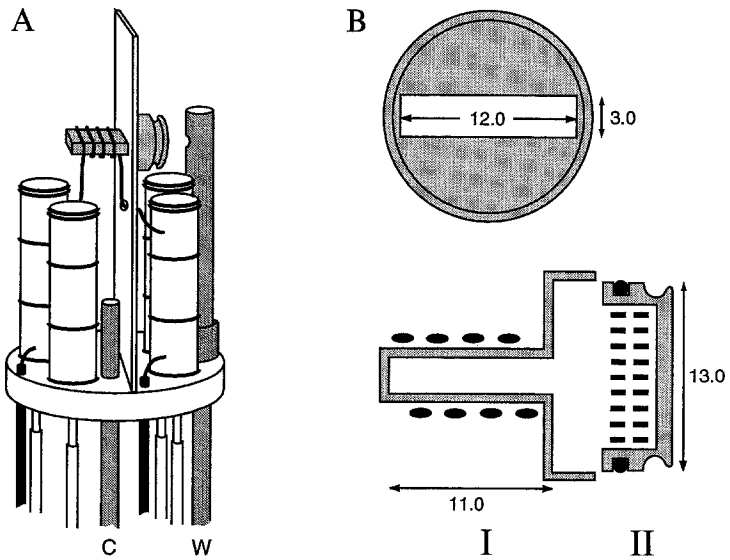

Fig. 1. Double-tuned (^{31}P/^{1}H or ^{15}N/^{1}H) VT flat-coil NMR probe for controlling the hydration of oriented phospholipid bilayer samples in solid-state NMR studies. (A) 44 mm o.d. probe, including the combined sample/hydration housing; and (B) cross sections of the housing. Regulated cool and warm air streams are supplied through the two tubes marked C and W (upper parts produced from Torlon) to the sample and hydration chamber, respectively. The two sections of the probe are separated by a shield of teflon, as shown in (A).

equilibration of hydration of the membrane sample from the reservoir.

To overcome the temperature-gradient problem, the probe was reorganized to allow selective variable-temperature (VT) control of the sample and hydration chamber. A 1 mm thick sheet of teflon inserted between the two chambers serves to separate the two parts of the probe affected by a cool and a warm air stream, as illustrated in Fig. 1A. Selective heating of the reservoir to 36 °C, while applying an air stream at 23 °C to the sample compartment, provides efficient hydration from the reservoir; no changes in mobility and orientation of phospholipid membrane samples were observed following five days of rf pulsing.

All NMR probe tests were performed on a Varian XL-300 spectrometer (7.05 T, 51 mm bore magnet) with single 90° pulse excitation of ^{31}P (121.7 MHz) and ^{1}H decoupling (300 MHz) during acquisition. An acquisition time of 20 ms followed by a relaxation delay of 2 s, i.e., a decoupler duty cycle of 1%, was employed for most experiments. A ^{1}H decoupling field strength of 50 kHz was generated, applying about 25 W of rf power to the high-Q circuit of the probe. Since probe tuning is strongly affected by possible dehydration of the phospholipid sample, two in-line bidirectional couplers (Narda Microwave Corp., Model 3020A) were used to supervise forward and reflected power of the two rf transmitters during the experiments. For the same reason, the original Varian 6–8 dB decoupler transistor amplifier (solid-state NMR 'booster'), which is highly sensitive to reflected power, was replaced by an ENI (Electronic Navigation Instruments) Model 5100L, 50 dB gain, 100 W broadband amplifier and appropriate attenuators and filters. The phospholipid samples were prepared by placing 10 µl of a solution of DMPC (Avanti Polar Lipids, Alabaster) in methanol (0.5 mg/µl, 7 µmole) on each of the 15 glass plates. The plates were dried overnight at ambient pressure, then 4 h under vacuum, hydrated for five days above deionized water in a desiccator, and finally stacked immediately before the NMR experiments.

The control of the hydration of a phospholipid membrane sample achieved during an NMR experiment, using the present VT flat-coil NMR probe design (Fig. 1), is demonstrated in Fig. 2. A series of ^{31}P NMR spectra, 64 accumulations each, of two identical hydrated and oriented DMPC samples were recorded at different times during a long period of rf pulsing using the 1% decoupler duty cycle described above and illustrate (Fig. 2) the state of the samples after 0.5 (A), 36 (B), 44 (C) and 120 h (D) of the start of pulsing. The left column shows spectra obtained using the air-tight sample chamber without a wet cotton plug, while the right column depicts the corresponding spectra resulting from the hydration conditions described above with a cool (23 °C) and a warm (36 °C) air stream. The perfectly oriented spectra (e.g., observed for both experiments in Fig. 2A) show an intense narrow resonance at 28 ppm (line width ca. 1.6 ppm), corresponding to the $\sigma_{\parallel}$ position of the axially symmetric ^{31}P chemical shielding anisotropy (CSA) powder pattern partially averaged by molecular motion. A minor signal observed at −19 ppm corresponds to $\sigma_{\perp}$ for a small fraction of nonoriented material.

Comparison of the spectra in the two columns of Fig. 2 demonstrates that the dramatic change in mobility and orientation observed for the sample without hydration control (left column) can be completely prevented, at least for a period of five days, using the experimental set-up outlined in Fig. 1. Without hydration control, the gradual displacement in the ^{31}P resonance position observed du-

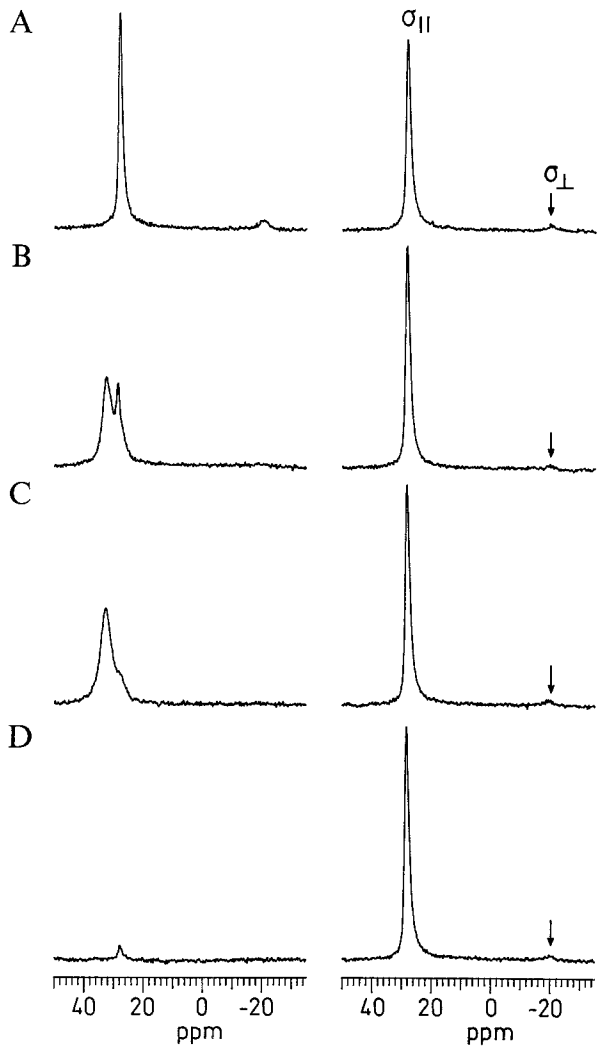

Fig. 2. Proton-decoupled ^{31}P flat-coil NMR spectra of oriented DMPC, obtained without (left column) and with (right column) VT hydration control using the air-tight sample chamber (Fig. 1B) in both cases. The spectra, 64 accumulations each, are recorded at times of (A) 0.5, (B) 36, (C) 44 and (D) 120 h after the start of continuous rf pulsing (1% decoupler duty cycle). Cool (23 °C) and warm (36 °C) air streams were supplied to the sample and hydration chamber, respectively, for the spectra in the right column. The ppm scale is relative to 85% H_3PO_4.

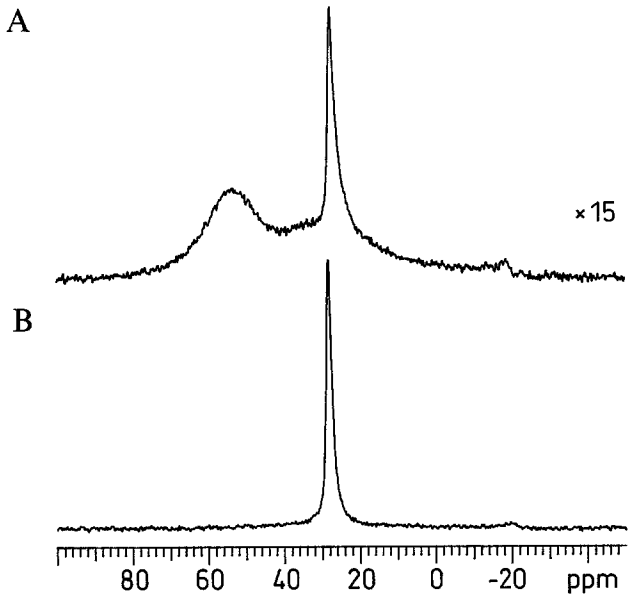

Fig. 3. Proton-decoupled ^{31}P flat-coil NMR spectra of oriented DMPC, obtained after 120 h of rf pulsing (conditions as in Fig. 2). The spectra were recorded using the air-tight sample chamber (A) without VT hydration control (4500 scans) and (B) with VT hydration control (64 scans). The vertical scale in (A) is expanded by a factor 15 relative to (B).

ring the first 12–36 h reveals a nonuniform decrease in mobility within the sample as a result of dehydration. The shift of the $\sigma_{\parallel}$ resonance towards higher frequency (Fig. 2B, left) occurs because the overall width of the motionally averaged ^{31}P CSA powder spectrum increases with the decrease in mobility. The concurrent increase in line width (Figs. 2B–D, left) indicates that the sample also undergoes changes in orientation. Indeed, the ^{31}P signal in Fig. 2D (left) is almost lost in broad anisotropic powder patterns, due to a complete deterioration of the sample. This is especially evident by comparison of the vertically expanded (×15) spectrum in Fig. 3A (the same spectrum as in Fig. 2D, left, except that the number of accumulations has been increased to 4500 to improve the signal-to-noise ratio) with that in Fig. 3B (identical to the spectrum in Fig. 2D, right).

In conclusion, a VT flat-coil NMR probe, capable of controlling hydration of oriented phospholipid bilayer samples in the course of long-term experiments, has been constructed. The design is expected to be of particular importance in studies of the structural organization of peptides and proteins oriented in lipid bilayers. It is noted that the hydration chamber also allows the use of different salt solutions to achieve specific relative humidities within the sample chamber for a variety of NMR studies using lipid membranes.

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