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Magnetic ordering of phospholipid membranes

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Phospholipid bilayers could be homogeneously oriented by application of strong magnetic fields as is demonstrated by means of deuterium and phosphorus nuclear magnetic resonance. The membranes were composed of either *Escherichia coli* lipids or a mixture of synthetic phosphatidylethanolamine and phosphatidylglycerol. The planes of the bilayer were found to align parallel to the magnetic field.

Diamagnetically anisotropic molecules can be oriented by an external magnetic field. The extent of orientation is small for single, small molecules but is enhanced dramatically for domains of interacting particles [1]. Almost quantitative magnetic ordering has been observed for nematic and lyotropic liquid crystals [2,3], concentrated solutions of biopolymers such as bacteriophages [4,5] and protein micro-crystals [6,7], and for membrane fragments which are rich in α-helical proteins [8–10]. Here we describe the first examples of a magnetic alignment of pure phospholipid membranes. The membranes were composed of either the total phospholipid extract of *Escherichia coli* membranes or of a mixture of *cis*-unsaturated synthetic phospholipids. At high water content ($\geq$ 85 wt% H_2O) almost perfect alignment of the bilayer domains with the planes of the bilayers parallel to the magnetic field (field strength 7 Tesla) was observed in less than 1 min for samples in the liquid crystalline phase. This effect may be helpful to prepare oriented lipid membranes for a variety of experimental purposes.

Nematic liquid crystals are widely used as solvents for nuclear magnetic resonance (NMR) spectroscopy of small molecules. Magnetic ordering of the nematic mesophase greatly simplifies the NMR spectra of the solute and allows an analysis of the molecular structure [2]. Likewise, the magnetic alignment of biopolymers has led to the elucidation of molecular structure by solid state NMR methods [11,12] as well as to a distinct improvement of the X-ray and neutron diffraction patterns [9,13]. The present observation that certain phospholipid membranes can easily be oriented by magnetic fields may bear on these points.

The magnetic alignment is demonstrated here by means of phosphorus and deuterium magnetic resonance. Fig. 1 shows ^{31}P-NMR spectra of a total lipid extract of *E. coli* cells as a function of temperature. The *E. coli* strain T 131 GP employed in this study was a glycerol-auxotroph with a simplified phospholipid composition of 80 wt% phosphatidylethanolamine (PE) and 20 wt% phosphatidylglycerol (PG) [14]. The fatty acid composition was that of wild-type *E. coli*. The lipids were dispersed in buffer (0.1 M NaCl/10 mM Pipes/1 mM EDTA (pH 7.0)) and hydrated without shaking at room temperature for several hours. The samples were then homogenized by gentle rotation followed by low speed centrifugation. The result-

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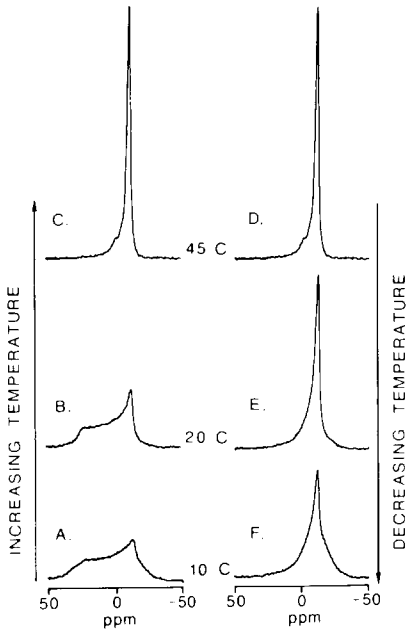

Fig. 1. ^{31}P-NMR spectra (at 121.4 MHz) of lipids extracted from *E. coli* and dispersed in buffer (88%). The approximate lipid composition is 80% PE and 20% PG. All spectra were obtained with 600 acquisitions and a recycle delay of 3 s. The spectra were all plotted with absolute intensity. The lipids in the gel phase give rise to typical powder spectra (A and B). Upon heating to 45°C the phospholipid bilayers align perpendicular to the magnetic field resulting in spectra C and D. Cooling this sample shows that the orientation is retained in the gel phase (E and F).

ing pellet was used for the NMR measurements. An essential prerequisite for uniform magnetic alignment was a water content of at least 80 wt%.

Figs. 1A and 1B represent ^{31}P-NMR spectra of a freshly prepared sample at 10°C and 20°C, respectively, where the membranes were not yet oriented. This is evidenced by the shapes of the spectra which are characteristic of a random distribution of bilayer orientations ('powder-type' spectra) with an axially symmetric chemical shielding tensor [15]. The latter arises from a fast rotation of the phosphate group around the bilayer normal as the axis of motional averaging. A chemical shielding anisotropy of $\Delta\sigma \simeq -45$ ppm, as defined by the separation of the edges of the powder-pattern, is typical for *E. coli* phospholipid membranes above and below the phase transition [16]. When this lipid sample is heated to 45°C the ^{31}P-NMR powder pattern is transformed into a single sharp resonance of increased intensity, positioned at the 90° edge of the powder pattern (Fig. 1C). This can only be explained by a macroscopic alignment of the bilayer membranes such that the individual domains become oriented with their normals perpendicular to the applied magnetic field. The asymmetry at the base of the resonance is indicative of some residual disorder, presumably due to boundary effects caused by the glass wall. The temperature dependence of membrane orientation can be explained by the phase behavior of *E. coli* PE which undergoes a broad gel-to-liquid crystal phase transition centered around 37°C [16]. A magnetic ordering is only possible when the membranes are in the fluid-like state. Figs. 1D–F further demonstrate that once magnetic ordering has been achieved at 45°C, the long-range order persists when the sample is cooled in the magnet to the starting temperature of 10°C. At temperatures below the phase transition the reorientation rate of the phosphate groups is greatly reduced which accounts for the homogeneous line broadening observed in both the comparison of Figs. 1A with 1B and 1E with 1F. Therefore, the sample appears to have completely retained the orientation achieved above the phase transition. As a result it seems likely that with a judicious choice of sample container that this sample could be mechanically turned in the field so that the bilayer normal could be oriented parallel with the field facililtating a number of structural studies.

Magnetic alignment was also achieved with membranes composed of synthetic lipids. In order to mimic the lipid composition of the *E. coli* membranes we have investigated mixtures of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE). POPG and POPE have gel-to-liquid crystal phase transition temperatures of −5°C and 26°C, respectively, which may be compared with −10°C for *E. coli* PG and 37°C for *E. coli* PE. The synthetic lipids give rise to much sharper thermodynamic transitions than the *E. coli* lipids. The individual lipids alone could not be oriented in the magnetic field. However, an almost perfect alignment was observed for a mixture of 83 wt% POPE and 17 wt% POPG dispersed in 85 wt% buffer. Figs. 2A and B represent ^{31}P-NMR spectra of this mixture where A corresponds

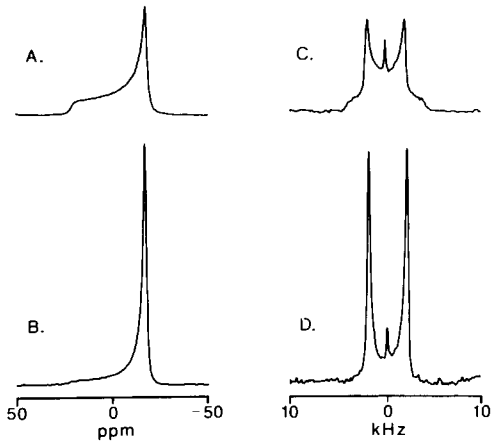

Fig. 2. NMR Spectra of a mixture of synthetic lipids; 83% POPE and 17% POPG dispersed in 85% buffer. POPG was selectively deuterated at the *sn*-2′ position of the glycerol head group. All spectra were recorded at 25°C and plotted with absolute intensity corrected for the number of acquisitions. A and B are ^{31}P-NMR spectra (at 121.4 MHz) obtained with 400 acquisitions and a recycle delay of 2 s, 50 Hz of line broadening was applied. C and D are ^{2}H spectra (at 46.1 MHz), C was obtained with 90000 acquisitions and D with 30000 acquisitions using a recycle delay of 250 ms, 90 Hz of linebroadening was applied. Spectra B and D demonstrate the orientation of the lipid bilayer by the magnetic field while A and C result from vortexing the sample and show typical powder patterns.

to the oriented sample while B results from vortexing the sample and shows a typical powder pattern. A similar comparison is made with ^{2}H-NMR spectra of the same sample displayed in C and D. The ^{2}H-NMR signal arises from POPG which was selectively deuterated at the *sn*-2′ position of the glycerol head group [17]. Spectrum C is characteristic of aligned membranes with essentially two sharp resonances while D represents the ^{2}H-NMR powder pattern for axially symmetric motions [18]. The resonance position in the aligned spectra coincide with the 90° edges of the powder pattern providing additional support for the orientation indicated above, i.e. with the planes of the membranes parallel to the magnetic field.

The origin of the magnetic orientation may be traced back to the diamagnetic anisotropy of the alkyl chains. Experiments with crystals of stearic acid have shown that the maximum diamagnetism is along the direction of the length of the molecule with a diamagnetic anisotropy of $\Delta\chi \simeq -25 \cdot 10^{-6}$ [19]. This is not sufficient to allow an orientation of an individual molecule but when large numbers of alkyl chains are held together parallel to one another the negative $\Delta\chi$ results in a tendency of the optical axis to align perpendicular to the applied magnetic field [20]. In contrast, the biological membranes which have been oriented so far were found to align with their optical axis parallel to the magnetic field. Apparently, the parallel α-helices of the proteins with their positive $\Delta\chi$ override the influence of the lipids [10].

The effect of magnetic fields on phospholipid membranes has been studied previously with optical birefringence methods [1,21]. For these investigations planar multilayers of phospholipid were prepared by shear between two glass plates. A linear dependence of the optical birefringence on the square of the magnetic field strength was observed. From the rather large value of the Cotton-Mouton-effect it was concluded that large intermolecular correlations within some kind of domains must be present.

The observed orientation of phospholipid membranes shows that a liquid crystalline array has been formed which is oriented by the very weak diamagnetic interactions between the fatty acyl chains and the static magnetic field of the NMR spectrometer. Because the orientation of the bilayer normal is perpendicular to the field and rapid motions occur about the bilayer normal, it is not possible to obtain direct structural information from these samples other than the measurement of the perpendicular component of axially symmetric powder patterns. However, the demonstration of a phospholipid sample that is affected by the small diamagnetic anisotropy of the fatty acyl chains provides an ideal matrix for orienting membrane soluble proteins and peptides with larger diamagnetic anisotropies which will orient the bilayers such that the bilayer normal is paralell with the field. These samples even with motional freedom about the normal axis will be useful for obtaining structural information from the orientation dependence of the nuclear spin interactions observed in solid state NMR experiments. Similarly, these oriented membranes may be useful for neutron diffraction studies.

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