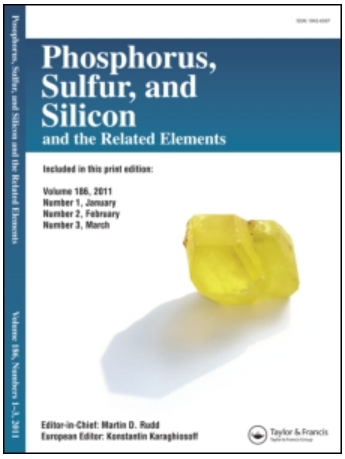

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^{31}P-NMR Methods for Investigating Phospholipid-Based Molecular Structure and Dynamics

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^{31}P-NMR METHODS FOR INVESTIGATING PHOSPHOLIPID-BASED MOLECULAR STRUCTURE AND DYNAMICS.

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Abstract: Phospholipids embedded in lamellar, hexagonal or isotropic phases, exhibit very different ^{31}P-NMR spectra. Phase changes induced by temperature, pH or addition of melittin or cholesterol can thus be proben. On the other hand, motions may drastically alter the spectral lineshapes and can be characterized by measuring NMR relaxation times. Correlation times and activation energies for intra-, inter-molecular and collective motions present in model membranes can thus be obtained.

INTRODUCTION

One of the basic components of biological membranes is the phospholipid molecule. Its peculiar amphipatic character insures a well known molecular organization, the bilayer membrane, giving to the cell its entity and functionality. This membrane is able to adapt to many physico-chemical constrains during the cell life (fusion, endocytosis, exocytosis, transport accross the membrane, etc...) by modifications of the basic bilayer organization. This mainly due to the wide variety of molecular organizations pure phospholipid bilayers can adopt depending on molecular structure and electrical charges at the phosphate level.

Phosphorus-31 solid state Nuclear Magnetic Resonance (^{31}P-NMR) is a particularly well suited method to follow the structure and dynamics of the molecule and of the supramolecular organization in which the phospholipid is embedded[1]. ^{31}P-NMR lineshapes are sensitive to phospholipid structuration whereas measurement of nuclear relaxation times affords to quantitate the dynamics.

THEORETICAL BACKGROUND

The phosphorus atom has only one naturally abundant isotope, ^{31}P, with non-zero nuclear spin. In addition, there is very often only one phosphorus per phospholipid which leads to a considerable simplification of NMR spectra. In these conditions, the dominant local magnetic interactions are the anisotropy of the chemical shift and the proton-phosphorus dipolar coupling usually suppressed by proton decoupling[2].

The spectrum lineshape obtained for powder samples, *i.e.* molecules oriented at random in the magnetic field, is governed by an angular dependent frequency, $\nu(\alpha,\beta)$:

$$\nu(\alpha,\beta) = \nu_0\left[1-\sigma_i-(\sigma_{xx}\cos^2\alpha\sin^2\beta+\sigma_{yy}\sin^2\alpha\sin^2\beta+\sigma_{zz}\cos^2\beta)\right] \quad (1)$$

where α and β are the Euler angles defining the orientation of the principal axis system of the chemical shift tensor, σ, with respect to the magnetic field direction, B_0. The σ_{kk} (k=x,y,z) stand for the principal elements of the tensor, σ_i for the isotropic chemical shift and ν_0 for the Larmor frequency. The ^{31}P-NMR lineshape decribed by Eq. (1) is shown in Figure 1A, in which the σ_{kk} are clearly seen and are taken from a static phospholipid powder sample, *i.e.* a phospholipid membrane at very low temperature[3].

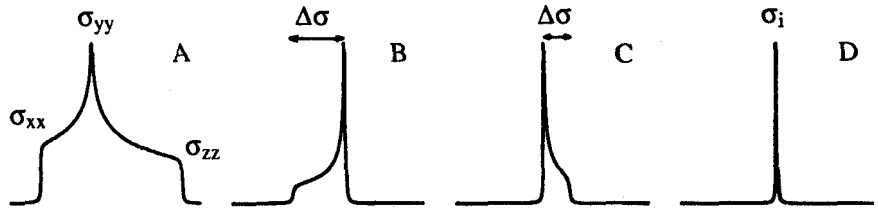

FIGURE 1 ^{31}P-NMR lineshapes: static (A), axial symmetry (B,C), isotropic (D)

At room temperature where fast motions appear in the membrane, this lineshape may be drastically altered. An axial rotation around the molecular long axis will lead to Figure 2B and a further rotation around an axis perpendicular to this latter will bring Figure 1C. Such lineshapes are governed by[1]:

$$\nu(\vartheta) = \nu_0\left[(1-\sigma_i - \frac{2}{3}\Delta\sigma\,(\frac{3\cos^2\vartheta-1}{2})\right] \qquad (2)$$

where ϑ is the angle between the motional axis and B_0, and $\Delta\sigma$ the residual chemical shift anisotropy. Variations of $\Delta\sigma$ are related to membrane ordering properties[3]. Fast isotropic tumbling will average $\Delta\sigma$ to zero and conduct to isotropic lines (Figure 1D).

Measurement of nuclear relaxation times T_{1Z} and T_{2E} affords the correlation times of fast and slow motions, respectively[3]. T_{1Z} is sensitive to motions in the range of the reciprocal of the Larmor frequency, ν_0. Employing high magnetic fields ($\nu_0 \approx$100-500 MHz), allows to probe dynamics in the range 10^{-11}s to 10^{-7}s. T_{2E} sensitively reflects processes with correlation times equal to the inverse of the chemical shift anisotropy ($\Delta\sigma \approx$ 1-50 kHz) thus offering a means to study the dynamical range 10^{-7}s to 10^{-2}s.

MEMBRANE STRUCTURE

The Figure 2 depicts nicely the power of ^{31}P-NMR for probing phase changes and membrane alteration upon addition of effectors. Figures 2A and 2B show the spectra of dimyristoylphosphatidylcholine (DMPC) in lamellar gel and fluid phases, respectively. The wider gel phase spectrum clearly indicates a more restricted lipid head group environment. Addition of 30 mole % cholesterol to this system completely abolishes the

above mentioned difference (Figures 2C and 2D), and reflects the desordering effect promoted by the sterol on gel phase lipids[4]. Addition of the bee venom toxin melittin to gel phase dipalmitoylphosphatidylcholine (DPPC) induces an isotropic line (Figure 2E) reflecting the membrane fragmentation into small isotropically tumbling objects[5]. For temperatures at which DPPC is in fluid phase, these objects fusion together into large vesicles producing a spectrum with non zero $\Delta\sigma$ (Figure 2F). The pH of acidic membranes can be well monitored by following dimyristoylphosphatidic acid (DMPA) gel phase spectra. DMPA spectrum at pH 8.4 (Figure 2G) is almost twice wider than at pH 4.2 (Figure 2H). Finally, egg phosphatidylethanolamine (Egg PE) spectra exihibit the well known lamellar (Figure 2I) to hexagonal (Figure 2J) phase transition on increasing the temperature. The additional rotation around the cylinder axes of the hexagonal phase, indeed leads to (with Eq. (2)) : $\Delta\sigma^{hexagonal} = -\Delta\sigma^{lamellar}/2$.

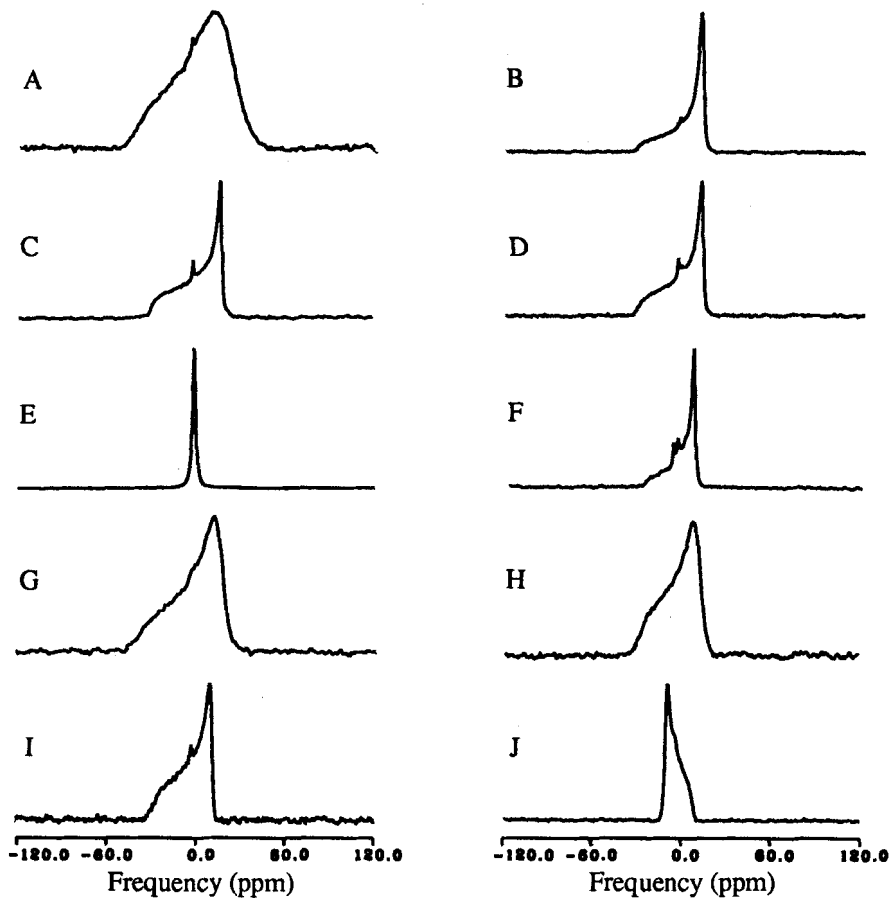

FIGURE 2 ^{31}P-NMR spectra of: DMPC in gel (A) & fluid (B) phases; DMPC with 30 mole % cholesterol (C) & (D); 6 mole % melittin on DPPC gel (E) & fluid (F) phases; DMPA in gel phases at pH 8.2 (G) & 4.2 (H); Egg PE at 25°C (I) & 40°C (J).

MEMBRANE DYNAMICS

Measurement of ^{31}P-NMR relaxation times T_{1Z} and T_{2E}, as a function of temperature and orientation of phospholipid bilayers with respect to the magnetic field, B_0, affords, in the frame of a suitable motional model, the correlation times and activation energies of the various motions active in these NMR time windows[3]. The Figure 3 depicts the temperature variation of the correlation times, τ_i, of the three classes of motions detected in DMPC bilayers. Among the three intramolecular motions τ_1, τ_2, τ_3 present at low temperatures (gel $L_{\beta'}$ phase), only the head group rotation, τ_1, is detected at high temperatures, the others being too fast to be proben in the NMR time scale. Intermolecular motions, *i.e.* molecular rotation, $\tau_{//}$, and wobbling, $\tau_{\perp}$, exhibit a considerable correlation time decrease on crossing the main phase transition. On the other hand, the collective motions associated to membrane hydrodynamics and characterized by a distribution of correlation times in the range 10^{-8}s to 10^{-2}s only appear in the fluid L_{α} phase. Slopes in Figure 3 lead to activation energies and show higher values for intermolecular motions indicating their more restricted character.

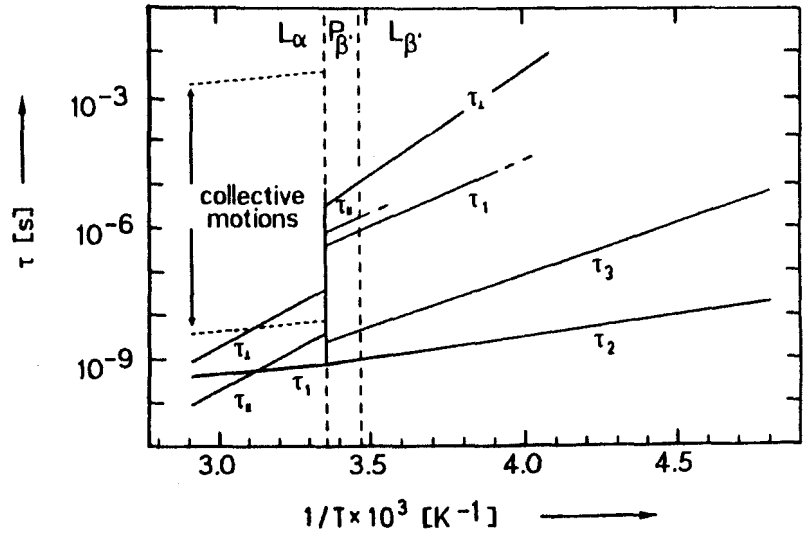

FIGURE 3 Thermal dependence of motional correlation times in DMPC bilayers[3]. See text for details.

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