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# HIGH PRESSURE 1D AND 2D NMR EXPERIMENTS ON MODEL BIOMEMBRANES

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We developed a high-pressure high-resolution probe for NMR experiments on biological systems which enabled us to study the phase behaviour of model biomembranes by 1D and 2D NMR experiments at temperatures up to 65°C and at pressures up to 3000 bar.

KEY WORDS: NMR, high pressure, model biomembranes

## 1. INTRODUCTION

The great interest in understanding biomembrane structure and function has stimulated intensive investigations of model membrane systems. A common feature of phosphatidylcholine model membranes is the existence of reversible gel ($L_{\beta'}$, $P_{\beta'}$) to liquid-crystalline (LC) phase-transitions. Besides the thermotropic phase-transitions, further pressure induced phase-transitions were observed by different methods, such as high-pressure Raman- (Wong, in Van Eldik, Jonas,eds., 1987) and neutron-scattering (Winter et al., 1989 ) experiments. As is well known, NMR-spectroscopy is a very powerful technique for studying structure and dynamics of biomembrane systems. Therefore, we tested the performance of our high-pressure high-resolution probe for 1D-and 2D-NOESY $^1$H-NMR experiments on the high-pressure phase behaviour of model biomembranes with the phospholipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC).

## 2. EXPERIMENTAL

Small unilamellar vesicles of 0.09M DMPC were prepared by ultrasonic irradiation of the lipid dispersed in $D_2O$. The $^1$H-NMR spectra were recorded at 180 MHz in a home-built NMR spectrometer. The high-pressure high-resolution probe used is shown in Fig. 1. The basic design consists of a titanium presssure vessel, that is closed at the top with a closure plug, which contains the rf and thermocouple leadthroughs. A box containing the rf resonant circuit is mounted directly to the plug. Attached to the bottom of the closure plug is a Vespel sample holder, which contains the saddle coil. The sample cell is placed in the sample holder. It consists of a modified 10mm NMR glass tube that is sealed to a PTFE bellow. The vessel is

pressurized with fluid $CS_2$, which is separated from the sample by the PTFE bellow. The temperature is controlled by circulating water through a thermostating jacket around the pressure vessel. The resolution of the non-spinning sample in this high-pressure set-up is 2 Hz.

## 3. RESULTS AND DISCUSSION

Fig. 2 shows as an example a 1D proton spectrum together with the peak assignment and a 2D-NOESY spectrum of DMPC at 64 °C and 700 bar, i.e. in the LC state of DMPC. One can clearly see the presence of intense cross-peaks between individual resonances which demonstrates the quality of the spectra under high pressure conditions. The cross-peak intensities increase drastically with increasing pressure from 1 bar to 2000 bar. A qualitative phenomenological interpretation of these data adopting the treatment of Xu, Cafiso (1986) reveals that the order parameter of the acyl chains increases with pressure from about 0.36 at 1 bar to about 0.6 at 2000 bar. This interpretation agrees very well with recent findings from high-pressure neutron-scattering experiments on DMPC, which showed that the bilayer thickness increases drastically with increasing pressure due to the large lateral compressibility of the membrane which leads to an enhancement of the chain order-parameter (Winter et al., 1989). Above 2200 bar at 64 °C drastic changes of the NMR spectra and relaxation times of DMPC indicate the pressure induced transition from the LC to the gel state, thus revealing, that valuable information about the location of high-pressure phase-transitions occuring in these systems can be obtained by these methods, too. Further high-pressure NMR studies of selectively deuterated lipids will help to determine even pressure/volume effects, correlation times and order-parameters of particular groups within the bilayer membrane system.

## REFERENCES

Van Eldik R., Jonas J. (eds,), High Pressure Chemistry and Biochemistry, D.Reidel Publ., NATO ASI C 197, 1987.

Winter R., Pilgrim W-C.,Ber.Bunsenges.Phys.Chem. 93, 708 (1989).

Xu Z.-C., Cafiso D.S., Biophys. J. 49, 779 (1986).

FIGURES Fig.1: The high-resolution high-pressure NMR probe.

A Box containing rf resonance circuit

B Closure plug

C Sample cell holder

D Sample

E Titanium pressure vessel

F PTFE separator

G High-pressure inlet

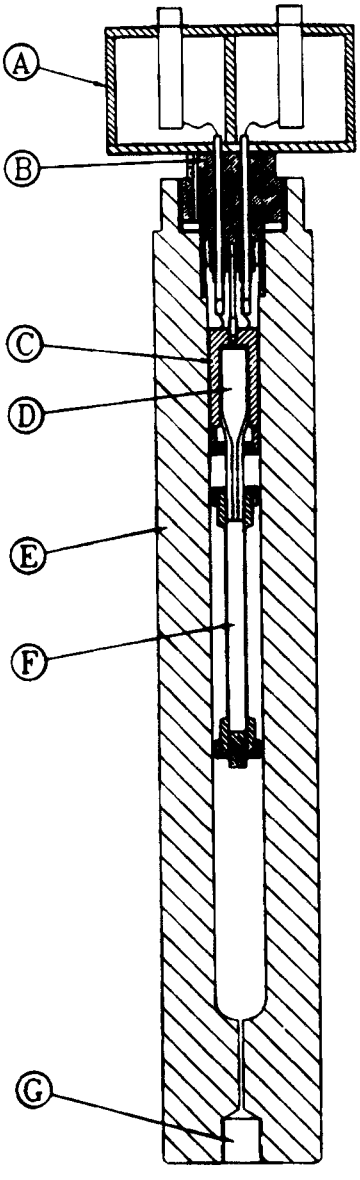


Fig.1

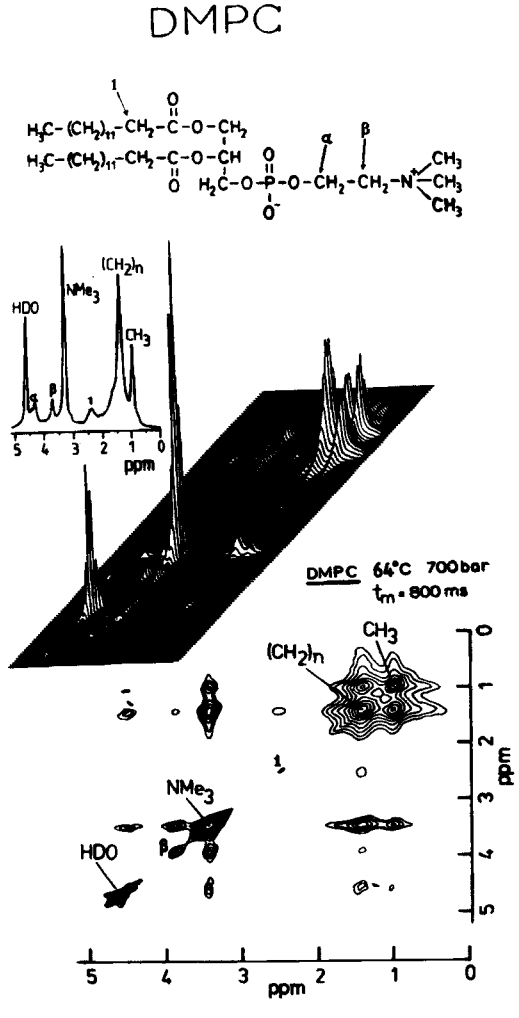


Fig.2 Proton NMR spectrum of DMPC and 2D-NOESY spectrum of DMPC at 64°C and 700 bar. The spectral assignment and chemical shifts are given on top of the figure.