Hydration Properties and Structure of Phosphatidylcholine Membranes in the Presence of *n*-Nonyl Bromide

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Abstract. Interaction of chemical fusogen *n*-nonyl bromide with a model membrane formed from phosphatidylcholine was studied using ^{2}D-NMR spectra of heavy water and ^{31}P-NMR proton decoupled spectra of the lipid phosphate group in multilamellar lipid dispersions. *n*-Nonyl bromide was found to influence the hydration layer of the model membrane. No participation of phosphatidylcholine molecules in non-bilayer configurations of the membrane was observed.

Key words: Membrane fusion — *n*-alkyl bromides — Structured water — ^{2}D-NMR — ^{31}P-NMR

Introduction

Mason et al. (1979, 1980) have reported that *n*-alkyl bromides can bring about fusion of cell membranes without affecting cell viabilities, membrane constitution, or ion transport; these compounds are also capable of inducing the fusion of phosphatidylcholine liposomes. The molecular mechanism of the membrane fusion induced by these novel fusion agents is unknown. Since the adhesion stage of the membrane fusion requires, in principle, local dehydration of the membrane surface (Rand 1981; Verkleij 1984) as demonstrated in the case of the well-known fusogen polyethylene glycol (Arnold et al. 1982), dehydration of the membrane is expected also in presence of *n*-alkyl bromides. In the joining and fission stage of fusion, the membrane bilayers in contact should pass an intermediate non-bilayer configuration (Lucy 1970; Ahkong et al. 1975). Evidence for this could be the formation of non-lamellar phases in different model membrane preparations under influence of polyethylene glycol (Boni et al. 1981), lipid fusogens (Cullis and Hope 1978; Hope and Cullis 1981) and other fusogens (for a review see Verkleij 1984).

In the present work we have investigated the effects of *n*-nonyl bromide on the hydration properties and structure of phosphatidylcholine multilamellar vesicles.

We have used ^{31}P-NMR spectroscopy for the detection of the possible non-bilayer structures in the membranes and ^{2}D-NMR spectroscopy for the investigation of their hydration properties.

Materials and Methods

Egg yolk phosphatidylcholine (EYPC) prepared according to Singleton et al. (1965) was stored in chloroform-metanol under N_2 at −30 °C. For sample preparation, the solvents were evaporated from known aliquots of lipid in a rotary evaporator. Possible traces of solvents were removed by evacuation at 1.5×10^{-3} Pa for 18 hours. Heavy water (D_2O) and *n*-alkyl bromides were added to egg PC with a microsyringe and the tubes were sealed. The samples were homogenized by repeated forth-and-back centrifugation and several freeze-thaw cycles. The samples were then stored at −30 °C. Before the measurements, the samples were equilibrated at room temperature for 12—24 hours. The composition of the samples during preparation was controlled gravimetrically.

All of the used chemicals were of analytical purity grade, the organic solvents were redistilled immediately before the use. D_2O was of 99.5 % isotopic purity and was used as obtained from V/O Izotop (Moscow, USSR). The purity of lipid was checked by thin-layer chromatography.

The NMR spectra were recorded on a Bruker HX-90 spectrometer with the Fourier transformation. ^{2}D-NMR spectra were measured at 6.49 MHz, 20 μs pulse width, and 700 μs pulse distance. ^{31}P-NMR spectra were measured at 36.43 MHz, a pulse width of 6 μs, a pulse distance of 700 ms, under inverted gated proton-noise decoupling using a home-build 600 W unit.

Results and Discussion

Below water concentration of 25 moles D_2O per mole of EYPC, the ^{2}D-NMR signals of all the prepared samples were split into a doublet. As described extensively in our previous paper (Národa et al. 1983), this splitting arises from the fact, that interaction of the quadrupolar moment of the ^{2}D nucleus with electric field gradient tensor of the D_2O molecules, bound to the lipid molecules in a model membrane, shifts the energy levels of the Zeeman interaction. In the case of the rapid ^{2}D exchange between different binding sites, the observed quadrupolar splitting, Δ, is then given by formula $\Delta = \sum_i p_i \Delta_i$, where p_i is the probability to find given ^{2}D nucleus in the binding site i characterized by the intrinsic quadrupolar splitting Δ_i. If there are different hydration shells of the lipid molecules in the model membrane with different intrinsic quadrupolar splittings, then the observed quadrupolar splitting is given by

$$\Delta = \left[\frac{1}{n}\sum_{i=1}^{j-1} n_i^0(\Delta_i - \Delta_j)\right] + \Delta_j \qquad (1)$$

where n is the $[D_2O]$:[lipid] molar ratio, Δ_i and Δ_j are the intrinsic quadrupolar splittings for i-th hydration shell containing n_i^0 D_2O molecules and for j-th shell containing n_j^0 D_2O molecules, respectively. Therefore, it is possible to deduce the values of n_i^0, n_j^0, Δ_i, Δ_j from the experimental data studying the experimental

values of Δ as a function of n. At water concentration above 25 moles of D_2O per mole of EYPC, superposition of two ^{2}D-NMR signals was observed (see Fig. 1). Besides the doublet described above, a central peak corresponding to the isotropically tumbling D_2O molecules was observed in all prepared samples. Intensity of this central peak increases with the increase of D_2O concentration, suggesting formation of the separated water phase in the studied systems with the properties of bulk water.

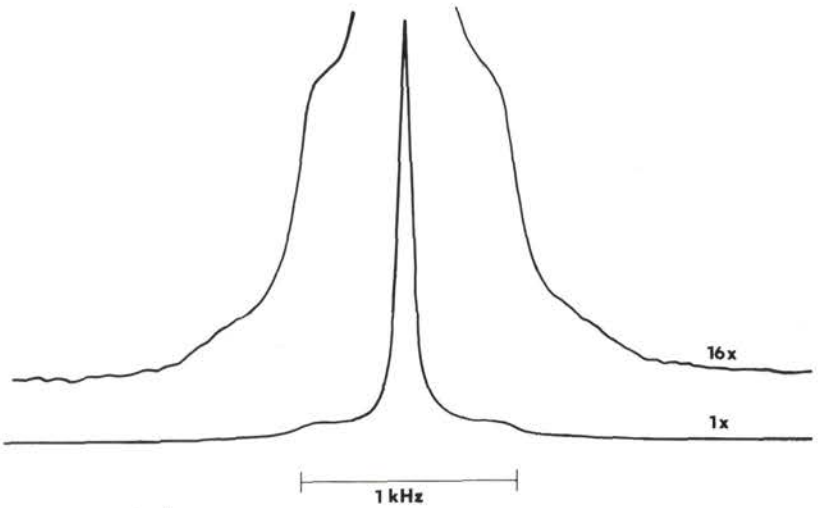

Fig. 1. A typical ^{2}D-NMR spectrum of an EYPC model membrane with *n*-nonyl bromide and excess D_2O. Molar ratios: [EYPC]:[*n*-nonyl bromide] = 4:1 and [EYPC]:[D_2O] = 1:80.

Evaluation of the experimental data according to equation (1) at fixed molar ratio of [*n*-nonyl bromide]:[EYPC] = 1:4 and at different water concentrations showed that besides the bulk water phase there are two different shells of D_2O in this system (see Fig. 2). The first hydration shell contains $n_1^0 = 11 \pm 1$ D_2O molecules per lipid. The tumbling of D_2O molecular in this shell is anisotropic, and the intrinsic quadrupolar splitting calculated according to equation (1) is $\Delta_1 = 450 \pm 20$ Hz. For the same hydration shell in the pure EYPC model membrane we found $n_1^0 = 12 \pm 1$ and $\Delta_1 = 370 \pm 20$ Hz. The second shell occurs at $12 < n < 25$ molar ratio, and the intrinsic splitting is $\Delta_2 = 0 \pm 20$ Hz for both studied systems, both with and without the added *n*-nonyl bromide. This second shell has, therefore, properties of water trapped between hydrated (by the first water shell) bilayers. The motion of the trapped water is isotropic ($\Delta_2 \approx 0$ Hz) but the values of $\Delta \neq 0$ within $25 > n > 12$ indicates a rapid D_2O exchange with the first hydration shell. Since the observed quadrupolar splitting Δ at $n > 25$ (where the bulk water phase is formed) is independent of water concentration ($\Delta = 480 \pm 20$ Hz for

[EYPC]:[*n*-nonyl bromide] = 1:4, and $\Delta = 430 \pm 20$ Hz for the pure EYPC membrane), the D_2O exchange between the separated bulk water phase and the first hydration shell is slow on the NMR time scale. The values of quadrupolar splitting Δ at $n > 25$ were dependent on the [EYPC]:[*n*-nonyl bromide] molar ratio, increasing with the increase of *n*-nonyl bromide concentration.

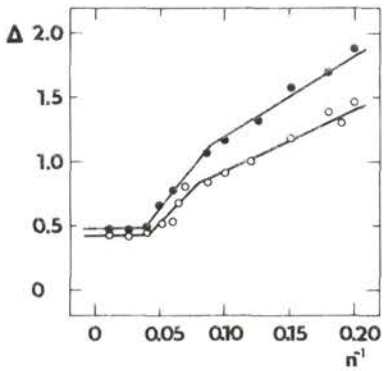

Fig. 2. Dependence of the quadrupolar splitting, Δ, on the molar ratio n^{-1} = [EYPC]:[D_2O]. Open symbols: [EYPC]:[*n*-nonyl bromide] = 1:0. Full symbols: [EYPC]:[*n*-nonyl bromide] = 4:1. Δ is given in kHz.

All the described observations suggest that *n*-nonyl bromide affects the first hydration shell of the model EYPC membrane, increasing the intrinsic quadrupolar splitting Δ_1 in this shell. We tested whether the increase of Δ_1 could be attributed to the conformation changes in the polar region of the EYPC molecules in membranes. An experimental technique suitable for the observation of these changes is ^{31}P-NMR spectroscopy (Balgavý et al. 1984). Figure 3 shows a sequence of ^{31}P-NMR spectra recorded at increasing [EYPC]:[*n*-nonyl bromide] molar ratio. The appearence of the spectra is typical of the lamellar lipid phase, with anisotropic EYPC motion, and chemical shift anisotropy is independent of *n*-nonyl bromide concentration and equals $\Delta\sigma_{eff} = -43 \pm 1$ ppm. Independence of $\Delta\sigma_{eff}$ on *n*-nonyl bromide concentration indicates that the influence of the studied fusogen on the EYPC polar fragment conformation is very small.

Noteworthy is the absence of non-bilayer phase signals in the ^{31}P-NMR spectra. It is possible that the half-lives of the intermediate structures are quite short or comparable with the ^{31}P-NMR time scale to be observed in ^{31}P-NMR spectra as suggested by Siegel (1984) for inverted micellar intermediates in fusion. However, there also is another possibility that the intermediate non-bilayer structures could form without the participation of phospholipids, as a separated phase of *n*-nonyl bromide molecules.

Phase separation of *n*-alkanes in lipid bilayers has already been observed by

several groups of authors (Dilger 1981; Croasmun 1980; Pope et al. 1984) using different experimental techniques, such as optical reflectance measurements of bilayer thickness of planar (black) membranes or ^{2}D-NMR spectroscopy of ^{2}D-labeled alkanes in phosphatidylcholine — water dispersions. It was suggested that alkanes separate in the form of microlenses which coalesce over the time into

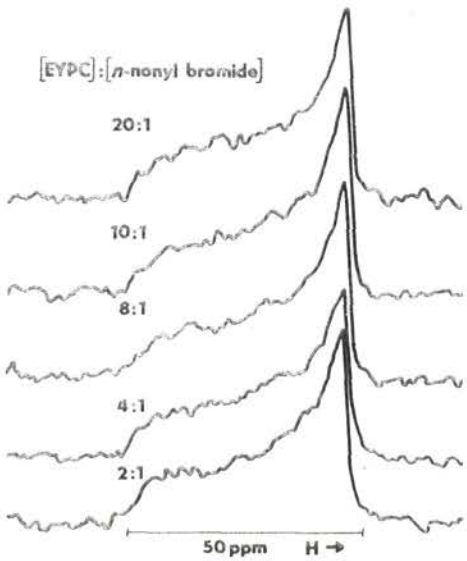

Fig. 3. Proton decoupled ^{31}P-NMR spectra of the EYPC model membrane at various *n*-nonyl bromide concentrations. [EYPC]:[D_2O] = 1:80.

larger aggregates. We suppose that similar phase separation occurs in bilayers also in the presence of *n*-alkyl bromides. Our experimental results do not contradict such a possibility, moreover, formation of the separated alkyl bromide phase within the bilyer might be responsible for the observed increased intrinsic quadrupolar splitting without the involvement of EYPC head group conformation changes.

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