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The effect of polysialic acid on molecular dynamics of model membranes studied by ^{31}P NMR spectroscopy

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Abstract

The paper reports the results of our study on the dynamics of model phospholipid membranes studied by ^{31}P NMR spectroscopy. The ^{31}P NMR spectra of multilamellar vesicles in temperatures below the main phase transition of PC are reported. The ^{31}P NMR spectra revealed changes caused by an increase of the membrane fluidity when polysialic acid (polySia) was applied as a modifying agent. The presence of polySia in the external environment of the phospholipid vesicles changes the motional freedom in the region of phosphate group of lipids. Increase of polysialic acid concentration changes structural properties of a membrane by increasing its fluidity.

Keywords: Polysialic acid; Phosphatidylcholine bilayer; Multilamellar liposomes; Molecular dynamics; ^{31}P–NMR

1. Introduction

PolySia is a term used to refer to linear homopolymers of $\alpha(2,8)$–sialic acid residues displayed at the surface of some mammalian cells. Polysialic acid bears multiple negative charges and is heavily hydrated [1]. PolySia takes a part in the several cell processes, which are not explained yet. This acid exists as a virulent determinant in the neuroinvasional bacterium such as *Escherichia coli K1* and *Neisseria meningitidis*. It masks O-antigens on the bacterium cell surfaces making easier the colonisation process of these bacteria in the suckling brain. Polysialic acid is typically linked to neural cell adhesion molecule N-CAM and controls the embryonic forms of N-CAMs in early evolution of embryo [2–6]. The level of polymerisation of polySia on the N-CAM molecules is the critical parameter for the normal morphogenesis and evolution of the nervous system. The expression of polySia into the cancer cell can develop the forces of cancer distribution (polySia play role as antigen). It was shown that a presence of polySia in the external environment of the phospholipid liposomes can change the molecular dynamics of the model membrane [7]. Nuclear magnetic resonance is a powerful experimental technique able to shed a new light on many aspects of the lipid bilayer modification. ^{31}P NMR spectroscopy gives the information on changes in the structure and dynamics of the polar choline heads of phospholipids of the model lipid membrane [8–11]. The ^{31}P NMR spectra of multilamellar vesicles in the temperatures below the main phase transition of PC are reported. The spectra reveal changes caused by an increase of the membrane fluidity when polysialic acid (polySia) was applied as a modifying agent. An increase in the polySia concentration produces an effect similar to that observed by rising temperature. In both cases the motional freedom of phosphate group of lipid increases.

2. Materials and methods

2.1. Chemicals

Egg-yolk phosphatidylcholine (PC) and polysialic acid (polySia) were purchased from Sigma. Heavy water

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(D_2O) was obtained from the Institute of Nuclear Research, Świerk, Poland.

2.2. Preparation of multilamellar liposomes

The phosphatidylcholine (PC) was dried under nitrogen and dispersed in D_2O. The final concentration of phosphatidylcholine was 25 mg ml^{-1}. The suspension was mixed with polysialic acid (polySia) which concentration was 0%, 2%, 5%, 8% and 10%.

2.3. ^{31}P NMR

^{31}P NMR spectra were recorded on Varian Unity 300 MHz spectrometer operating at resonance frequency 121.4 MHz using broadband proton decoupling (WALTZ-16). Eighty five percent phosphoric acid was used as the external standard.

3. Results

Fig. 1 presents ^{31}P NMR spectra of PC multilamellar vesicles at different temperatures. The ^{31}P NMR spectrum is a superposition of the broad anisotropic line characteristic for lamellar phase of phospholipids and a narrow isotropic line, typical for the phospholipids with isotropic mobility [12]. Increase of the temperature results in the narrowing of the spectrum, which is indicative of the decrease of chemical shift anisotropy [13]. This effect may be interpreted as directly related to an increase of motional freedom of polar headgroups in phospholipid membrane, where a phosphorus atom is located. The effect of narrowing NMR spectrum was observed below the temperature of main phase transition. For pure PC, the observed decrease of the chemical shift anisotropy in temperature dependence is typical for multilamellar vesicles [14,15]. For the temperatures higher than 20°C the contribution

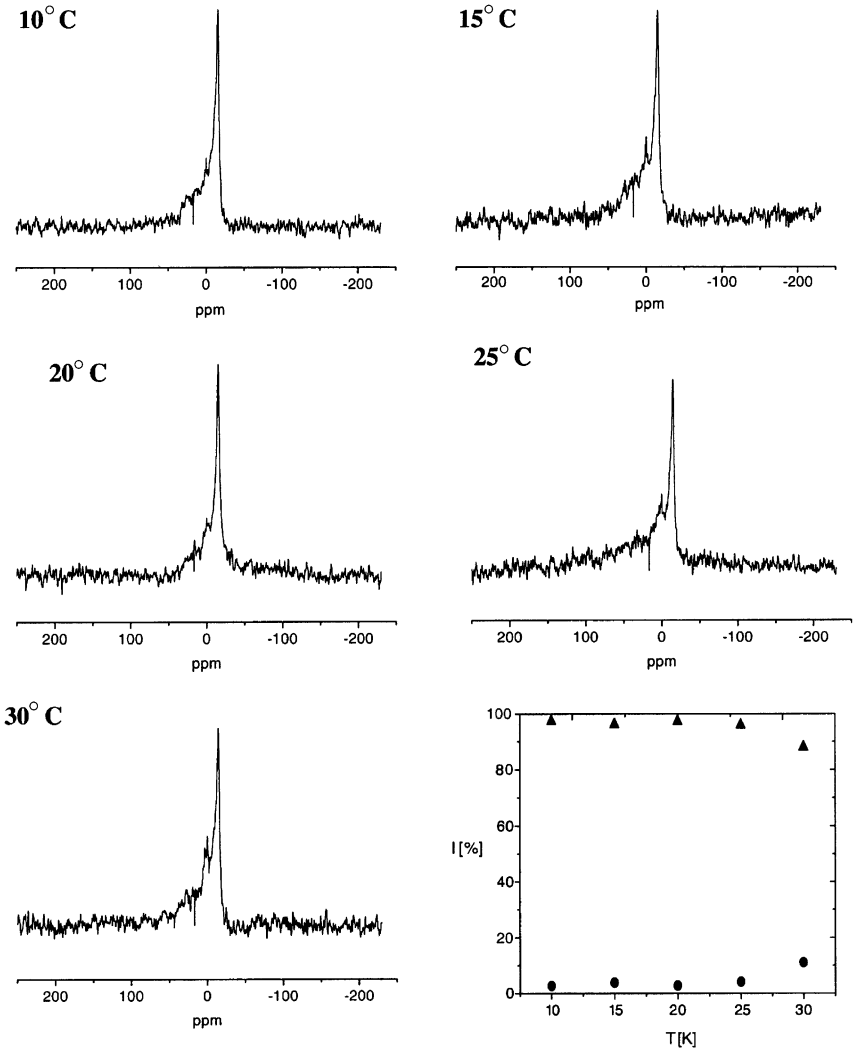

Fig. 1. ^{31}P NMR spectra of multilamellar vesicles of PC at different temperatures, as indicated. The calculated isotropic and anisotropic line contributions I (%) are given by circles and triangles, respectively.

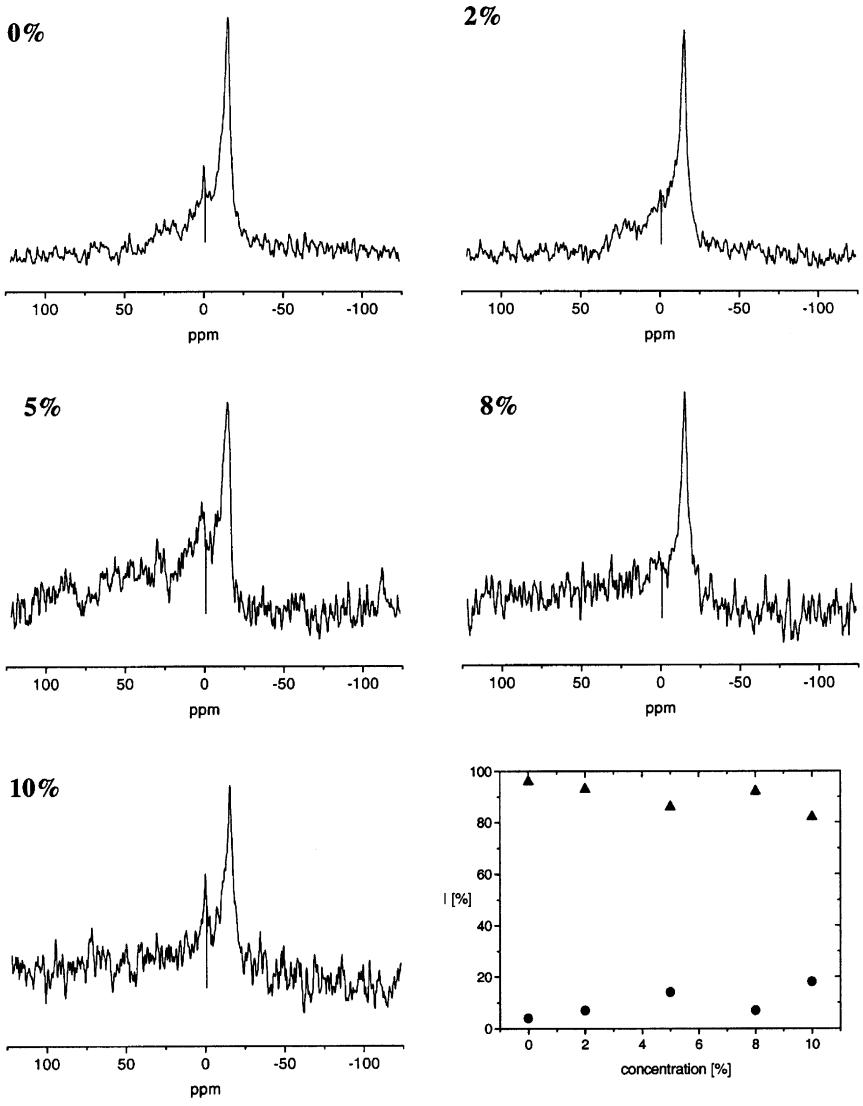

Fig. 2. ^{31}P NMR spectra recorded at 15°C of multilamellar vesicles of PC containing different molecular ratio of polySia, as indicated. The calculated isotropic and anisotropic line contributions I (%) are given by circles and triangles, respectively.

of isotropic lines increases. The calculated temperature dependence of contributions of isotropic and anisotropic lines I [%] is presented in Fig. 1.

Incorporation of polySia into PC membranes increases motional freedom of polar headgroups, an effect similar to a rising of temperature [16]. The ^{31}P NMR spectra (15°C) of different concentrations of polySia are presented in Fig. 2. Incorporation of polySia on PC multilamellar vesicles narrows anisotropic line. The calculated concentration dependence of the isotropic and anisotropic lines contributions I [%] is presented in Fig. 2.

4. Discussion

Physical properties of model PC multilamellar membranes are the result of lipid–lipid interaction (as in a one component system) and lipid–polySia interaction. ^{31}P NMR gives some insight into the nature of these interactions at the molecular level. Increase of polysialic acid concentration changes dynamical properties of a membrane [17]. The polar regions of polySia interact with polar heads of the PC multilamellar vesicles. ^{31}P NMR spectra of polySia and PC membranes indicate an increase of motional freedom of polar headgroups in a bilayer, where a phosphorous atom is located. Since polySia polar molecules are not likely to be present in the lipid core of the membrane (apolar region) due to repulsive forces between the lipid polar headgroup and the polySia, such result indicates some mismatch in the well-ordered model membrane structure [18–22].

The additional line broadening in the spectra of the phospholipid vesicles could arise from microscopic or macroscopic sample "heterogeneity" giving rise to distribution of chemical shift anisotropy values [20,23–25]. This additional

contribution can be function of time of measurement. Therefore the ^{31}P spectra are charged by high error. The calculated contributions of I (%) (Figs. 1 and 2) show rather qualitative (tendency of changes) than quantitative changes of I (%).

The reported here effect of polySia on dynamics of PC polar head in membrane contributes to the understanding of a possible mechanism of the two cellular processes. These processes appear to be affected by polySia during the formation of tissues, namely of guidance and targeting of growing axons, and the separation and migration of cells [1–3,6]. It is believed that in both situations the presence of polysialic acid on cells helps them to detach from their neighbours, thus allowing them to respond to guidance or targeting cells, or to exhibit the plastic interactions required for motility.

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