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# Polysialic acid can mediate membrane interactions by interacting with phospholipids

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ABSTRACT

Polysialic acid (polySia) is expressed on the surface of neural cells, neuroinvasive bacterial cells and several tumor cells. PolySia chains attached to NCAM can influence both *trans* interactions between membranes of two cells and *cis* interactions. Here, we report on the involvement of phospholipids in regulation of membrane interactions by polySia. The pH at the surface of liposomes, specific molecular area of phosphatidylcholine molecules, phase transition of DPPC bilayers, cyclic voltammograms of BLMs, and electron micrographs of phosphatidylcholine vesicles were studied after addition of polysialic acid free in solution. The results indicate that polySia chains can associate with phosphatidylcholine bilayers, incorporate into the polar part of a phospholipid monolayer, modulate *cis* interactions between phosphatidylcholine molecules, and facilitate *trans* interactions between apposing phospholipid vesicles. These observations imply that polySia attached to NCAM or to lipids can behave similarly.


## 1. Introduction

Polysialic acid (polySia) forms a structurally and functionally unique group of linear carbohydrates with a degree of polymerization (DP) up to ca. 200 sialyl residues (for review see Troy, 1992; Mühlenhoff et al., 1998; Janas and Janas, 2005; Rutishauser, 2008). PolySia is expressed on the cell surface of both bacterial and eukaryotic cells. The polySia capsule on the surface of neuroinvasive *Escherichia coli* K1 and *Neisseria meningitides* serogroup B and C cells is essential in pathogenesis, since it facilitates bacterial invasion and colonization of the meninges in neonates (Troy, 1992). The poor immunogenicity of the polySia capsule results from its structural identity to evolutionarily structures present on an embryonic neural cell adhesion molecule (NCAM) (Troy, 1992). PolySia serves also as an oncodevelopmental antigen in several tumor cells and may enhance their metastatic potential (Troy, 1992), and it was also found on unfertilized eggs of rainbow trout (Inoue and Iwasaki, 1978) and on a voltage sensitive sodium channel (James and Agnew, 1987).

The broadest expression of polySia in the nervous system occurs in the early phases of the tissue's development and its function can be described as a "slippery non-receptor" (Rutishauser, 2008). In embryonic vertebrates polySia chains are involved in precursor migration and axon outgrowth and targeting, in neonatal animals – in circuit maturation. Although overall polySia levels are substantially reduced in the adult brain, high levels of polySia persist in distinct regions that retain neurogenic activity (Rutishauser, 2008). In adult animals the polySia is required for activity-induced synaptic plasticity in central nervous system (CNS) (Gascon et al., 2007).

These functions of polySia come from its unique biophysical properties. At physiological pH, monomers of polySia are negatively charged due to the presence of carboxylic group ($pK_a = 2.6$) and negatively polycharged polySia chains can influence both *trans* interactions (between membranes of two opposite cells) and *cis* interactions (between NCAM and other proteins in the plane of plasma membranes containing polysialylated NCAM) (Rutishauser, 2008). PolySia chains have a high degree of hydration and the hydrated volume regulates the membrane–membrane distance and the receptor–receptor interaction (Rutishauser, 2008). Although the effect of polySia on membrane–membrane interaction was studied (Rutishauser et al., 1985, 1988; Johnson et al., 2005), only protein–protein interactions were discussed thus the possible involvement of phospholipids in regulation of membrane interactions by polysialic acid was not considered.

We applied biophysical techniques to evaluate the role of phospholipids both in *cis* interactions (within a phospholipid bilayer)

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and *trans* interactions (between two apposing phospholipid bilayers) mediated by polySia chains.

## 2. Materials and methods

### 2.1. Chemicals

Dipalmitoylphosphatidylcholine (DPPC) was purchased from Fluka. DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), egg-yolk phosphatidylcholine, sodium salt of polysialic acid (poly-α2,8-acetylneuraminic acid, sodium salt, mean DP = 70, from *E. coli* K1), and α(2 → 3,6,8,9)-Neuraminidase from *Arthrobacter ureafaciens* were purchased from Sigma. N-(5-Fluoresceinthiocaramoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (fluorescein-PE) and 1,6 diphenyl-1,3,5-hexatriene (DPH) were purchased from Molecular Probes. Sephacryl S-200 and S-300 were purchased from Amersham Pharmacia.

### 2.2. Purification and characterization of polySia

Since the phosphodiester bond by which the lipid is bound to the polySia is acid labile (Decher et al., 1990), the purchased polySia was treated at 60 °C for 1 h at pH 5 in order to remove any residual lipids. This procedure may induce a partial hydrolysis of polySia (Kakehi et al., 1996). Final purification was achieved by chromatography on Sephacryl S-300 and precipitation by 80% ethanol (Decher et al., 1990). The Sephacryl S-200 and S-300 gel filtration was used for the estimation of the average degree of polymerization (DP) of purified polySia using molecular weight markers (Kanato et al., 2008). The samples were analyzed using UV absorption at 200 nm (Cheng et al., 1998). A spectrum of chain lengths was detected with a maximum in the range of DP = 40–60, and with an average DP of ~50. This value can be compared with the literature data on cellular polySia chain lengths: the average chain length of endogenous polySia chains in *E. coli* K1 have been found to be ~165 residues/chain (Rohr and Troy, 1980); whereas most of the polySia chains on NCAM have consisted of DPs ~50–60 or less (Livingstone et al., 1988; Inoue and Inoue, 2001; Nakata and Troy, 2005), a subpopulations of chains with DPs over ~100 (Nakata and Troy, 2005; Galuska et al., 2008) and extending to DPs ~400 (Nakata and Troy, 2005; Drake et al., 2008) have been detected. The addition of this purified sodium salt of polysialic acid at indicated concentrations to the liposomal dispersion did not cause any measurable changes neither in bulk pH nor in the surface pressure of the air–water interface. This observation is in agreement with the results of Okubo and Kobayashi (1998) who showed that linear-type macroions (e.g. sodium chondroitin sulfates, sodium polyglutamate and DNA) have no surface activity in a wide range of biopolymer concentrations.

### 2.3. Measurement of pH at the membrane surface and calculation of polySia adsorption coefficient

Fluorescein-PE was used as a probe of pH at the lipid–water interface (McLaughlin and Harary, 1976; Langner et al., 1995). Large unilamellar vesicles (LUV) were produced in buffer A (1 mM KCl, 149 mM NaCl, 10 mM Tris–HCl, pH 7.13) by standard extrusion technique using polycarbonate filters (Nucleopore, 100 nm pore size) (Janas et al., 2004). The lipid content of samples was 0.07 mg/ml and the fluorescein-PE concentration was 0.5 mol% of the total lipid (egg-yolk phosphatidylcholine). Excitation and emission wavelengths were 496 nm and 519 nm, respectively. Calculation of polySia adsorption coefficient was performed according to Gouy–Chapman–Stern theory (McLaughlin and Harary, 1976; Langner et al., 1995). The concentration of protons at the membrane surface, $[H^+]$, depends on the surface charge density, $\sigma$, through the surface potential, $\psi_0$:

$$[H^+] = [H^+]_0 \exp\left(-\frac{F\psi_0}{RT}\right) \quad \text{and} \quad \psi_0 = \frac{s}{\varepsilon\varepsilon_0\kappa}$$

where $[H^+]_0$ is the bulk concentration of $[H^+]$, $F$ is Faraday constant, $R$ is gas constant, $T$ is the absolute temperature, $\varepsilon$ is a dielectric constant of the medium, $\varepsilon_0$ is the permittivity of free space, $\kappa = (2\bar{e}^2 z^2 Nc/\varepsilon\varepsilon_0 kT)^{1/2}$, $c$ is the total concentration of monovalent electrolyte in the bulk aqueous solution, $z$ is the valance of the ion, $\bar{e}$ is the elementary charge, $N$ is the Avogadro number, and $k$ is the Boltzman constant. The charge density at the surface of lipid bilayer, after adsorption of polySia, was calculated assuming a mean DP = 50 of one polySia chain. Lipid vesicles without fluorescein-PE were used to correct for light scattering.

### 2.4. Monolayer formation and isotherm recording

Monolayer experiments were performed as previously described (Janas et al., 2000b). The monolayers were deposited by spreading a proper volume of DOPC mixture in chloroform. Monomolecular layers at the air–water interface were formed in a 10 cm × 40 cm Teflon trough. The experiments were run at 24 °C. The humidity was kept at 85%. Prior to isotherm recording, monolayers were equilibrated at zero pressure for 5 min to allow evaporation of chloroform. Lipid monolayers were then compressed at a speed of 0.5 mm/s. Surface pressure was measured by tensiometer PS 3 (Nima Technology). Measurement error was less than 0.1 mN/m. Dionised water was used as the subphase. The experiments were performed both in the presence of polySia (0.5 mg/ml) and in the absence of polySia in the subphase.

### 2.5. Anisotropy measurements using fluorescence spectroscopy

Steady-state fluorescence anisotropy experiments on DPH in 100 nm DPPC liposomes (1:1000 mol ratio, probe:lipid) were performed as a function of temperature using a FL WinLab Perkin Elmer Luminescence Spectrometer LS 50B. Liposomes were produced by suspending the dried lipid mixture of DPPC (1 mg/ml) and phenylhexatriene (DPH) in water, then heating above the transition temperature of DPPC followed by extrusion. Excitation and emission wavelengths were 384 nm and 429 nm, respectively. LUV in buffer A were added to polysialic acid solution in buffer A at indicated final concentration of polySia. For control experiments the same volume of buffer A was used without polySia. Fluorescence anisotropy, $r$, was calculated as described (Janas et al., 2006).

### 2.6. Voltammetric measurements

Spherical bilayer lipid membranes (BLMs) were formed from the mixture of DOPC and hexadecaprenol in molar ratio 10:1 on a Teflon capillary tube in aqueous solution of 100 mM and 200 mM KCl, inside and outside the membrane, respectively (Janas et al., 2000a). In cyclic voltammetry method, a potential scan is applied between the working electrode and the reference electrode, and the current passing through the electrochemical cell is measured versus the applied potential. The potential scans were in the range −50 mV and +50 mV, the scan rate was 10,000 mV/s. The experiments were performed both in the presence and in the absence of polySia (0.5 mg/ml) outside membrane.

### 2.7. Transmission electron microscopy (TEM)

Lipid vesicles were prepared from DOPC both in the presence of polySia (0.5 mg/ml) and in the absence of polySia according to (Janas et al., 2000a). Small amounts of lipid in chloroform was dried