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ARTICLE

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Membrane transport of polysialic acid chains: modulation of transmembrane potential

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Abstract Polysialic acid (polySia) is a long polyanionic polymer (with the degree of polymerization, DP, up to 200) of negatively charged sialic acid monomers. PolySia chains are bound to the external surface of some neuroinvasive bacterial cells and neural cells. PolySia serves as a potent regulator of cell interactions via its unusual biophysical properties. In the present paper, the analysis, based on the Goldman-Hodgkin-Katz equation, of transmembrane potential changes resulting from transmembrane translocation of polySia is performed. The relationships between the transmembrane potential and the polySia DP (up to 200), the temperature, the cation/anion permeability ratio, and the inner/outer concentration ratio of polySia has been plotted and discussed. The maximal membrane potential changes, up to 118 mV, were found for a permeability ratio greater than one. The increase of the polySia chain length resulted in the diminution of this effect. The temperature-dependent changes in membrane potential were less than 7 mV in the range 0–50 °C. The change in the concentration ratio (into its reciprocal) resulted in a mirror reflection of the membrane potential curves. The results show that the expression of polySia chains in bacterial cells can be responsible for the modulation of the transmembrane potential of the bacterial inner membrane. We suggest that the polySia chains can influence the transmembrane potential of neural cell membranes in a similar way. This analysis also describes the effect of the transmembrane translocation of negatively charged polyanionic polynucleotydes on the cell membrane potential.

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Introduction

Polysialic acid (polySia) was first described in the culture filtrate of *Escherichia coli* K-235 (Barry and Goebel 1957). Subsequent studies showed polySia to be an oligosaccharide with a degree of polymerization (DP) of 10–12 sialyl residues and these oligomers of sialic acid appeared in the culture filtrate as a result of acid-catalyzed hydrolysis from membrane-associated polysialic acids of ca. 150–200 sialyl residues (Troy 1979). PolySia chains constitute a structurally unique group of carbohydrate residues that covalently modify surface glycoconjugates on cells that range in evolutionary diversity from bacteria to human brains (Troy 1992). Expression of the polySia capsule on the surface of neuroinvasive *E. coli* K1 and *N. meningitidis* serogroup B and C cells is important in pathogenesis, since it appears to facilitate bacterial invasion and colonization of the meninges in neonates. Little is known about how the capsule can influence the pathogenic character of these strains, except that its expression may represent an elaborate survival mechanism that evolved to trick the human immune system by masking the somatic O-antigen moieties of lipopolysaccharides. Finally, the capsule in *E. coli* K1 strains is a receptor for polySia-specific bacteriophages that require expression of the capsule for infectivity (Troy 1992). The DP in bacterial cells can extend beyond 200 polySia residues (Troy 1992). PolySia is an oncodevelopmental antigen in human kidney and brain, and may enhance the metastatic potential of Wilms tumor cells and neuroblastomas (Kiss and Rougon 1997). These novel carbohydrate chains also appear to have a regulatory role in cell growth, differentiation, fertilization and neuronal pathogenicity (Kiss and Rougon 1997). Studies on the function of polySia suggest that its primary role is to promote developmentally

controlled and activity-dependent plasticity in cell interactions and thereby facilitate changes in the structure and function of the nervous system (Rutishauser 1998). The initial recognition of polySia function in vertebrate development was through its ability to decrease the neural cell adhesion molecule (NCAM)-mediated membrane-membrane adhesion in vitro. Subsequently it was observed that a negative regulation of other cell interactions could also occur in the absence, or independent of, the NCAM-binding function. PolySia is associated with cell rearrangement and migration. The movement of cells involves the breaking of cell interactions, both in an initial detachment phase from their site of origin and in the adhesion-deadhesion process of locomotion along a substrate. An example of detachment occurs in the sorting out of skeletal muscle myotubes. PolySia appears also to be an important regulatory element in neural plasticity, nerve regeneration, and as an oncofetal marker on tumor cells (Rutishauser 1998). Although a lot of surface molecules regulate cell-cell interactions, polySia appears unique both in its simple structure and in the multitude and complexity of events it might control (Kiss and Rougon 1997).

As a linear homopolymer (DP ca. 8–200) of α-2,8-linked sialic acid, polySia is a remarkably simple macromolecule (Rutishauser and Landmesse 1996). The valency of this polyanion corresponds with the degree of polySia polymerization. The minimum chain length of polySia reflects the characteristic formation of a helical conformation (Michon et al. 1987). In neural cells, polySia is attached to neural cell adhesion molecule (NCAM), the only confirmed cell surface polySia carrier in vertebrates, through a developmentally regulated process. PolySia is able to attenuate adhesion forces and modulate overall cell surface interactions, thereby orchestrating dynamic changes in the shape and movement of cells, as well as their processes (Kiss and Rougon 1997). The concentration of polySia at the cell surface results from the combination of several regulatory mechanisms. One is biosynthesis, which probably depends on the level of transcription and/or activity of the polysialyltransferase. The other mechanisms are biosynthesis independent. They involve intracellular trafficking to and from the cell surface, as well as degradation. It can be speculated that the latter processes result in rapid changes of polySia surface expression, whereas changes in biosynthesis would be observable only after longer periods of time. Moreover, biosynthesis-independent changes might be transient and/or localized to some membrane subdomains, whereas those attributable to changes in biosynthesis should be long lasting (Kiss and Rougon 1997).

The transmembrane translocation of polysialic acid chains across the inner membrane of *E. coli* K1 bacterial cells requires both the membrane protomotive force and the transmembrane electrical potential gradient (Troy et al. 1990a, b, 1991). The genes encoding proteins necessary for synthesis and expression of the polySia capsule in *E. coli* K1 have been cloned and characterized (Boulnois et al. 1987). In *E. coli*, the 12–14 genes required for these processes are located in a multiple kps cluster. The 17 kb kps cluster is divided into three functional regions (Silver et al. 1981). The central region 2 contains the information for synthesis, activation, and polymerization of sialic acid. The region 3 genes are postulated to be involved in transport of polySia across the bacterial inner membrane, while region 1 genes appear to function in the transport of polymers to the external surface of the outer membrane.

In the present paper an equation has been derived showing the dependence of membrane potential on the degree of polySia polymerization, temperature, permeability ratio, and concentration ratio. This derivation is based on the Goldman-Hodgkin-Katz equation. The analysis of membrane potential changes resulting from transmembrane translocation of polySia is performed.

Derivation of the membrane potential equation

Let us consider the flow of ions across a neutral membrane of thickness h, enclosing a cell or a subcellular vesicular structure, thus separating two solutions (the outer and the inner). We take the x-axis perpendicular to the membrane, its surfaces being placed at $x=0$ and $x=h$ (Fig. 1, inset). Let $c=c^{(i)}$ for $x<0$, $c=c^{(o)}$ for $x>h$, and $V_m=V_{in}-V_{out}$, where c is the concentration of the ions, V_m is the transmembrane electrical potential, and V_{in} and V_{out} are the electrical potentials of the inner and outer solutions, respectively. Following Goldman (1943) and Hodgkin and Katz (1949), we assume that the electric field is constant within the membrane. Then from the Goldman-Hodgkin-Katz equation for positive ions we obtain:

$$J_+ = z_+ P_+ v_m \frac{c_+^{(o)} - c_+^{(i)} e^{z_+ v_m}}{1 - e^{z_+ v_m}} \quad (1)$$

where J_+ is the positive ions flux, z_+ is the positive ions valency ($z_+ = +1, +2, \ldots$), P_+ is the permeability coefficient of positive ions, v_m is the dimensionless potential normalized in units of kT/e, $c_+^{(o)}$ is the concentration of positive ions in the OUT compartment, and $c_+^{(i)}$ is the concentration of positive ions in the IN compartment.

From the Goldman-Hodgkin-Katz equation for negative ions we obtain:

$$J_- = z_- P_- v_m \frac{c_-^{(o)} - c_-^{(i)} e^{z - v_m}}{1 - e^{z_- v_m}} \quad (2)$$

where J_- is the negative ions flux, z_- is the negative ions valency ($z_- = -1, -2, \ldots$), P_- is the permeability coefficient of negative ions, $c_-^{(o)}$ is the concentration of negative ions in the OUT compartment, and $c_-^{(i)}$ is the concentration of negative ions in the IN compartment.

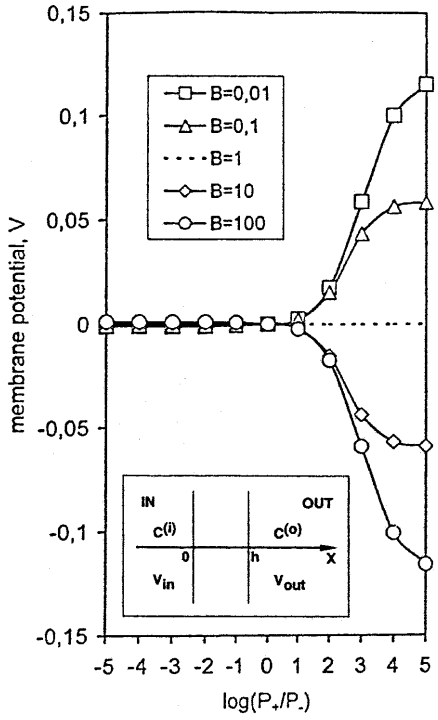

Fig. 1 The transmembrane potential, V_m, as a function of the ratio of the permeability coefficients, P_+/P_-. Each line was plotted for the constant ratio of the polysialic acid (polySia) concentration, $B = c_-^{(i)}/c_-^{(o)}$. Results calculated for the polySia ionic valency equal to –100 and a temperature of 298 K. P_+, P_- are the permeability coefficients for the univalent cation and the polyanion (polySia), respectively; $c_-^{(i)}, c_-^{(o)}$ are the concentrations of polySia in the inner and the outer solutions, respectively. *Inset*: a membrane of thickness, h, separating two compartments IN and OUT. V_{in} and V_{out} are the electrical potentials in the IN and OUT compartments, respectively; $c^{(i)}$ and $c^{(o)}$ are the concentrations of the solutions in the IN and OUT compartments, respectively

The relation between the transmembrane potential, V_m, and the normalized potential, v_m, is:

$$V_m = v_m \frac{kT}{e} \tag{3}$$

where k is the Boltzmann's constant, T is the absolute temperature, and e is the elementary positive charge.

The total current of ions passing through the membrane, i, can be written:

$$i = i_+ + i_- \tag{4}$$

where i_+ is the current of positive ions, i_- is the current of negative ions, and:

$$i = zFJ \tag{5}$$

$$i_+ = z_+FJ_+ \tag{6}$$

$$i_- = z_-FJ_- \tag{7}$$

In the stationary state, i equals zero and therefore $i_+ + i_- = 0$.

After combining Eqs. (5), (6) and (7) we obtain:

$$z_+FJ_+ = -z_-FJ_- \tag{8}$$

Let us assume that the ratio of the ion valencies is constant and equals α:

$$\alpha = \frac{z_-}{z_+} \tag{9}$$

Then from Eq. (8) we obtain:

$$J_+ = -\alpha J_- \tag{10}$$

As required by electroneutrality:

$$\sum |z_+|c_+ = \sum |z_-|c_- \tag{11}$$

where $|z_+| = z_+$, $|z_-| = -z_-$. For a single polyunivalent solute (e.g. a potassium salt of polysialic acid) on both sides of the membrane, we obtain:

$$z_+c_+^{(i)} = -z_-c_-^{(i)} \tag{12}$$

$$z_+c_+^{(o)} = -z_-c_-^{(o)} \tag{13}$$

and after transformations:

$$c_+^{(i)} = -\frac{z_-}{z_+}c_-^{(i)} \tag{14}$$

$$c_+^{(o)} = -\frac{z_-}{z_+}c_-^{(o)} \tag{15}$$

Combining with Eq. (9) we obtain:

$$c_+^{(i)} = -\alpha c_-^{(i)} \tag{16}$$

$$c_+^{(o)} = -\alpha c_-^{(o)} \tag{17}$$

Inserting Eqs. (1) and (2) into Eq. (10) we obtain:

$$z_+P_+v_m\frac{c_+^{(o)} - c_+^{(i)}e^{z_+v_m}}{1 - e^{z_+v_m}} = -\alpha z_-P_-v_m\frac{c_-^{(o)} - c_-^{(i)}e^{z_-v_m}}{1 - e^{z_-v_m}} \tag{18}$$

Then we divide the above equation by $v_m z_+$:

$$P_+\frac{c_+^{(o)} - c_+^{(i)}e^{z_+v_m}}{1 - e^{z_+v_m}} = -\alpha\frac{z_-}{z_+}P_-\frac{c_-^{(o)} - c_-^{(i)}e^{z_-v_m}}{1 - e^{z_-v_m}} \tag{19}$$

Substituting z_-/z_+ for the factor α we obtain:

$$P_+\frac{c_+^{(o)} - c_+^{(i)}e^{z_+v_m}}{1 - e^{z_+v_m}} = -\alpha^2P_-\frac{c_-^{(o)} - c_-^{(i)}e^{z-v_m}}{1 - e^{z-v_m}} \tag{20}$$

After inserting Eqs. (3), (16) and (17) into the above equation we obtain:

$$P_+\frac{-c_-^{(o)} + c_-^{(i)}e^{z_+\frac{V_m e}{kT}}}{1 - e^{z_+\frac{V_m e}{kT}}} + \alpha P_-\frac{c_-^{(o)} - c_-^{(i)}e^{\alpha z_+\frac{V_m e}{kT}}}{1 - e^{\alpha z_+\frac{V_m e}{kT}}} = 0 \tag{21}$$

Equation (21) has been used for further computational analysis.

Solving the membrane potential equation

A Mathematica (Wolfram Research) computational application was used for solving the membrane potential equation. It was impossible to obtain the explicit solution of Eq. (21); thus this equation was modified by introducing the following factors:

$$A = \frac{P_+}{P_-} \tag{22}$$

$$B = \frac{c_-^{(i)}}{c_-^{(o)}} \tag{23}$$

$$x = \exp(eV_m/kT) \tag{24}$$

Inserting these three variables into Eq. (21) and taking $z_+ = +1$, we get:

$$A(1 - Bx)(1 - x^{z_-}) = z_-(1 - Bx^{z_-})(1 - x) \tag{25}$$

Equation (25) was solved using variable x as an unknown quantity. The values of x were used for calculation of the transmembrane potential, V_m, in accordance with Eq. (24).

Results and discussion

In the present paper the analysis has been carried out of membrane potential changes as a function of the degree of polySia polymerization, temperature, the permeability ratio ($A = P_+/P_-$), and the concentration ratio $\left(B = c_-^{(i)}/c_-^{(o)}\right)$. In contrast to other papers, where similar investigations have been done for ions of the same valency (Ohshima et al. 1988), or a mixture of uniunivalent and diunivalent solute with identical anions (Ohki 1984), our work was performed for polyunivalent solute, with the polyanion valency ranging from 1 to 200. As shown in Fig. 1, the values of the membrane potential for a permeability ratio less than 1 and a polySia valency equal to 100 are less than ± 1 mV. The membrane potential reaches a value higher than 100 mV for a concentration ratio equal to 0.01 and a permeability ratio equal to 10^5. For a concentration ratio equal to 100 the shapes of the curves are symmetrical with negative values of the membrane potential. The influence of the concentration ratio on the membrane potential is more significant for a permeability ratio less than 1 (i.e. for the case when the permeability coefficient for polysialic acid is higher than the permeability coefficient for the univalent cation) and for a value of the polysialic acid ionic valency, z_-, equal to -100, in comparison with $z_- = -10$. For permeability ratio values greater than 1 [$\log(P_+/P_-) > 0$] the influence of the polysialic acid ionic valency on the membrane potential curves is most visible for a value of the permeability ratio equal to 10. In this case the increase of polySia ionic valency from –10 to –100 results in the decrease of the membrane potential from ± 15 mV to ± 2 mV (data not shown).

As shown in Fig. 2, the dependences of the membrane potential on the temperature are linear, with positive values of the slope for a permeability ratio less than 1, and with negative values of the slope for a permeability ratio greater than 1. In the temperature range from 0 to 50 °C the variations of the membrane potential are less than 1 mV for a permeability ratio less than 1. These variations are much larger for a permeability ratio greater than 1, up to 7 mV in the case of a permeability ratio equal to 100.

The curves showing the membrane potential as a function of the concentration ratio have a center of symmetry for a polySia valency equal to –100 (Fig. 3). For a polySia valency $z_- = -10$ the increase of the permeability ratio from 0.01 to 100 causes an increase of the membrane potential from –16 mV to +62 mV (for a concentration ratio equal to 10^{-5}) and a decrease of the membrane potential from +16 mV to –62 mV (for a concentration ratio equal to 10^5) (data not shown). For a polySia ionic valency $z_- = -100$ the changes of the membrane potential are much smaller: from –2 mV to +18 mV for a concentration ratio equal to 10^{-5} (i.e. for

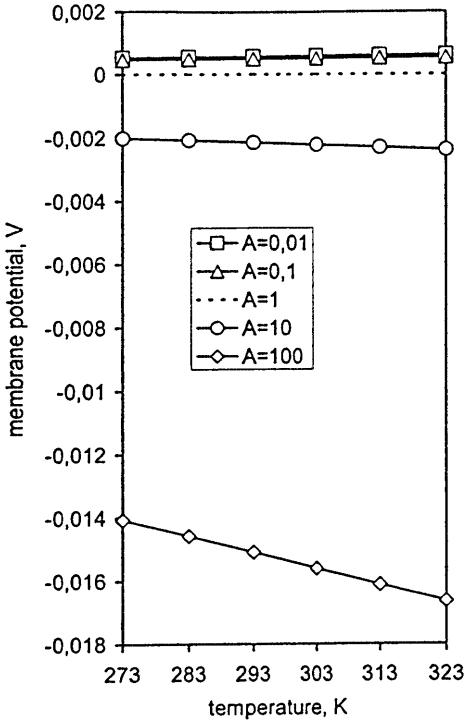

Fig. 2 The transmembrane potential, V_m, as a function of temperature, T. Each line was plotted for a constant ratio of the polysialic acid (polySia) permeability coefficients, $A = P_+/P_-$. Results calculated for the polySia ionic valency equal to –100 and the ratio of polySia concentrations $c_-^{(i)}/c_-^{(o)}$ equal to 10. P_+, P_- are the permeability coefficients for the univalent cation and the polyanion (polySia), respectively; $c_-^{(i)}$, $c_-^{(o)}$ are concentrations of polySia in the inner and the outer solutions, respectively

the case when the outer concentration of polySia is 10^5-fold higher than the inner concentration).

Figure 4 shows the membrane potential as a function of the polySia ionic valency and temperature, calculated for the permeability ratio equal to 10^{-2} and the concentration ratio equal to 10^{-1} (Fig. 4a) and 10 (Fig. 4b). The change of the concentration ratios [the conversion from $c_{-}^{(o)} = 10c_{-}^{(i)}$ to $c_{-}^{(o)} = 0.1c_{-}^{(i)}$] results in a change of the curve monotonicity: from a decreasing function (from –0.3 mV for $z_{-}=-200$ up to –60 mV for $z_{-}=-1$) to an increasing function (from +0.3 mV for $z_{-}=-200$ up to +60 mV for $z_{-}=-1$). The changes in the membrane potential are the biggest (up to 52 mV) for short oligomers (i.e. for the polySia ionic valency between the values of –1 and –8); for the ionic valency between the values –8 and –30 the changes are up to 5 mV, and for long polySia polymers (i.e. for the ionic valency from –30 to –200) the changes are less than 1 mV. The Arrhenius plot based on the data from Fig. 4a is linear and the membrane potential changes increase with increasing temperature (data not shown).

Figure 5 presents the membrane potential as a function of the degree of polySia polymerization (presented in negative values which correspond to the polySia ionic valency) for the ratio of the permeability coefficients, $A = P_{+}/P_{-}$, between 10^{-4} and 10^{4}. The membrane potential was calculated for the ratio of polySia concentrations, $B = c_{-}^{(i)}/c_{-}^{(o)}$, equal to 10^2 and 10^{-2} (for Fig. 5a and b, respectively). For a permeability ratio less than 1, the membrane potential increases (Fig. 6a) from +0.4 mV (for $A=10^{-1}$, $z_{-}=-200$) to +118 mV (for $A=10^{-4}$, $z_{-}=-1$), reaching values greater than +50 mV only for $z_{-}=-1$ and $z_{-}=-2$. The changes of the membrane potential values for polySia chains longer than 7 or 10 monomers for $A=10^{-1}$ or $A=10^{-4}$, respectively, are very small [$V_m\epsilon(0; 1)$ mV]. In contrast, for a permeability ratio greater than 1, the membrane potential increases (in negative values) from –1.2 mV (for $A=10$, $z_{-}=-200$) to –118 mV (for $A=10^4$, $z_{-}=-1$), reaching values greater (in negative values) than –50 mV for the following cases: $z_{-}=-1$ and $A=10^1$; $z_{-}\epsilon[-14; -1]$ and $A=10^2$; $z_{-}\epsilon[-150; -1]$ and $A=10^3$; $z_{-}\epsilon[-200; -1]$ and $A=10^4$. The change in the

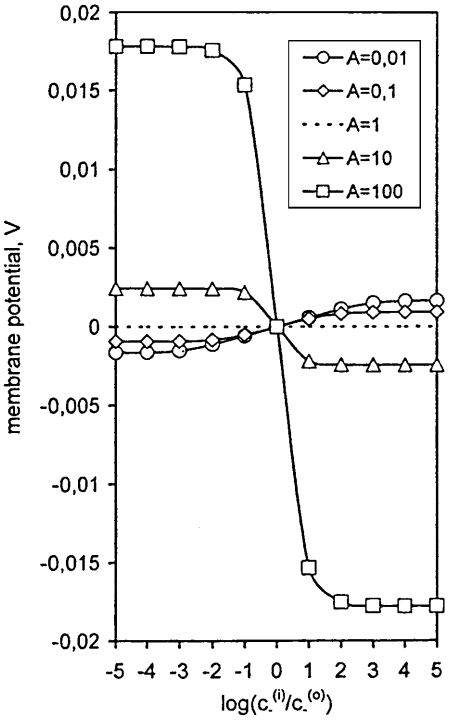

Fig. 3 The transmembrane potential, V_m, as a function of the ratio of the polysialic acid (polySia) concentrations, $c_{-}^{(i)}/c_{-}^{(o)}$. Each line was plotted for a constant ratio of the permeability coefficient, $A = P_{+}/P_{-}$. Results calculated for the polySia ionic valency equal to –100 and a temperature of 298 K

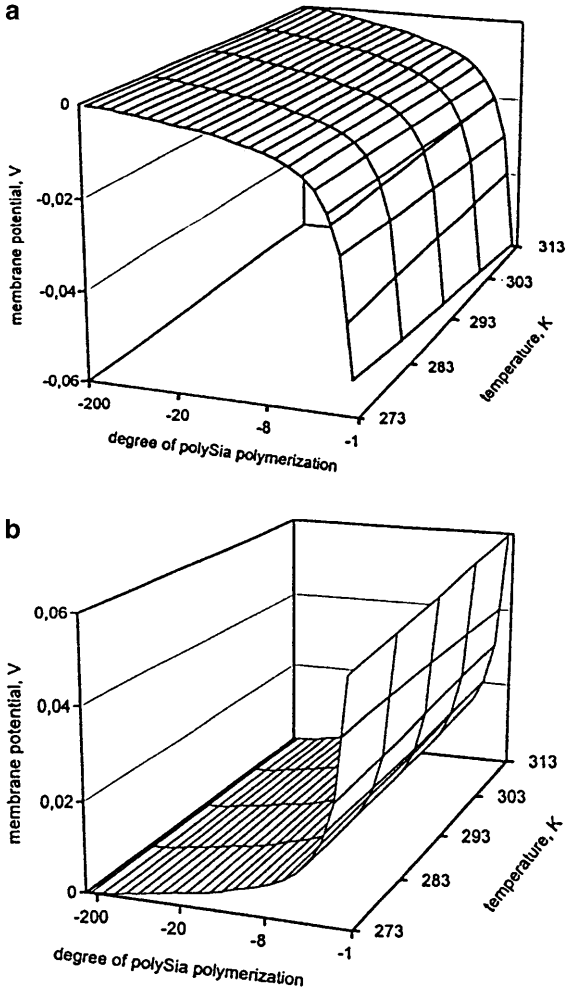

Fig. 4 **a** The transmembrane potential, V_m, as a function of the degree of polysialic acid (polySia) polymerization (presented in negative values which correspond to the polySia ionic valency, z_{-}) and the temperature. Results calculated for the ratio of the permeability coefficients, P_{+}/P_{-}, equal to 10^{-2} and the ratio of polySia concentrations, $c_{-}^{(i)}/c_{-}^{(o)}$, equal to 10^{-1}. **b** The transmembrane potential, V_m, as a function of the degree of polysialic acid (polySia) polymerization (presented in negative values which correspond to the polySia ionic valency, z_{-}) and the temperature. Results calculated for the ratio of the permeability coefficients, P_{+}/P_{-}, equal to 10^{-2} and the ratio of polySia concentrations, $c_{-}^{(i)}/c_{-}^{(o)}$, equal to 10

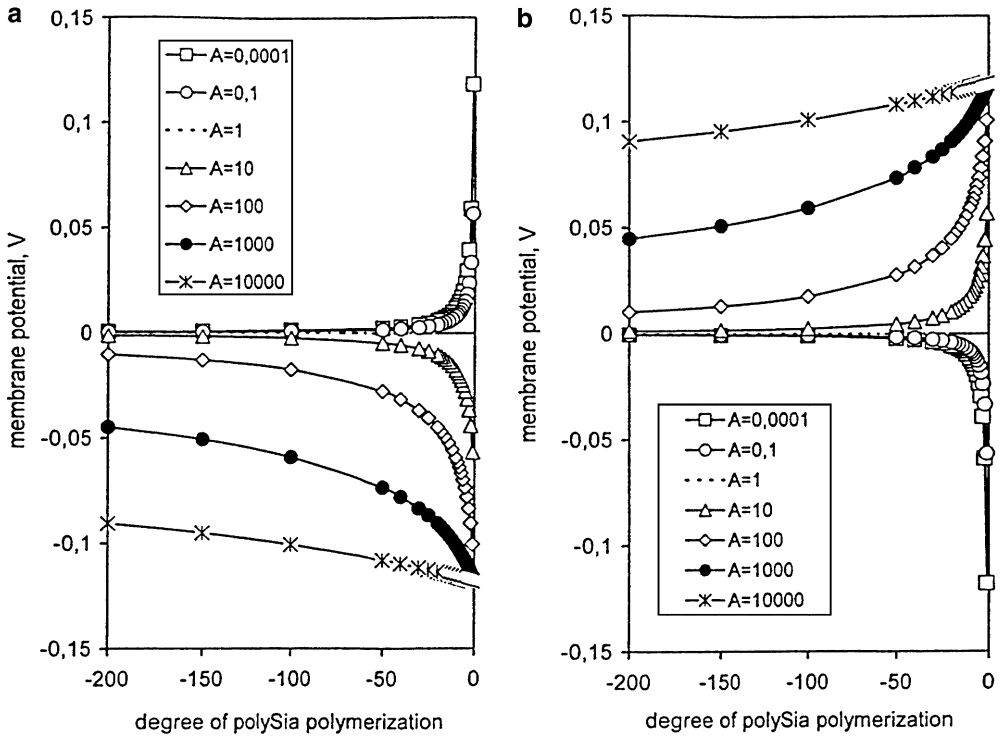

Fig. 5 **a** The transmembrane potential, V_m, as a function of the degree of polysialic acid (polySia) polymerization (presented in negative values which correspond to the polySia ionic valency, z_-). Each line was plotted for a constant ratio of the permeability coefficient, $A = P_+/P_-$. Results calculated for the ratio of polySia concentrations, $c_-^{(i)}/c_-^{(o)}$, equal to 10^2 and a temperature of 298 K. **b** The transmembrane potential, V_m, as a function of the degree of polysialic acid (polySia) polymerization (presented in negative values which correspond to the polySia ionic valency, z_-). Each line was plotted for the constant ratio of the permeability coefficient, $A = P_+/P_-$. Results calculated for the ratio of polySia concentrations, $c_-^{(i)}/c_-^{(o)}$, equal to 10^{-2} and a temperature of 298 K

concentration ratio [the conversion from $c_-^{(i)} = 10^2 c_-^{(o)}$ to $c_-^{(i)} = 10^{-2} c_-^{(o)}$] results in a mirror reflection of the membrane potential curves with respect to the x-axis (Fig. 5a and b).

As shown in Fig. 6a, for the permeability ratio, P_+/P_-, equal to 10^2, the membrane potential increases (in positive values) for a concentration ratio, $B = c_-^{(i)}/c_-^{(o)}$, less than 1. A similar tendency (in negative values of V_m) can be observed for a concentration ratio greater than 1. The shapes of the curves are symmetrical with respect to the x-axis. For a concentration ratio equal to 10^{-1}, the membrane potential increases from +10.4 mV to +118.0 mV [for $c_-^{(i)}/c_-^{(o)} = 10^{-4}$] with the polySia chain length decreasing from 200 monomers to 1. The corresponding increase of the membrane potential for the concentration ratio equal to 10^{-1} is smaller: from +9.2 mV to +56.7 mV. In the case of the conversion of the permeability ratios (from $P_+ = 10^2 P_-$ to $P_+ = 10^{-2} P_-$, Fig. 6b), the variations of the membrane potential are small [$V_m \epsilon (0.8; 5.3)$ mV] for long polymers (DP > 30) and for a concentration ratio equal to 10^4. For short polymers ($1 \leq DP \leq 8$) the changes of the membrane potential are much bigger: from 19.3 mV to 118.0 mV. The axial symmetry of the membrane potential curves, presented in Fig. 6a and b, is visualized in Fig. 6c. This figure shows the membrane potential as a function of the concentration ratio and the permeability ratio for the polySia chain length equal to 100 monomers. As can be seen, the value of the membrane potential is larger than 100 mV only for the concentration ratio and the permeability ratio equal to 10^{-4} and 10^4, respectively.

It was shown that the exact steady-state solution of the electrodiffusion equations for a simple membrane was the constant electric field solution when the ion environment was electroneutral on both sides of the membrane and the total numbers of ions of the same valency on both sides were equal (Arndt et al. 1970). In this paper, the equations for the transmembrane current density were derived based on the constant-field Goldman equation for a single uniunivalent solute on both sides of the membrane, two uniunivalent solutes with identical anions on both sides of the membrane, and two uniunivalent solutes and one diunivalent solute with identical anions on both sides of the membrane. In our investigations we consider a polyunivalent solute (a univalent cation and a polyanion) on both sides of the membrane.

Lewis (1979) used the Goldman-Hodgkin-Katz equation to test his experimental observations on the reversal potential and single-channel conductance of the acetylcholine-sensitive ionic channels at the neuromuscular junction. This equation formulated for for K^+, Na^+, and Ca^{2+} ions could not account for part of the experimental relationships. It seems from our results that the failure of the Goldman-Hodgkin-Katz equation to describe Lewis's experiments could result from the transmembrane translocation of polyvalent ions during the measurement.

The transmembrane potential, which may influence also other membrane properties, is equal to the sum of the cell resting potential and the difference of the inner and outer surface potential. The surface potentials are produced by surface fixed charges that also influence local concentrations of free ions (Burton 1995). The fixed charges on cell membrane surfaces are predominantly negative and cause a local accumulation of all

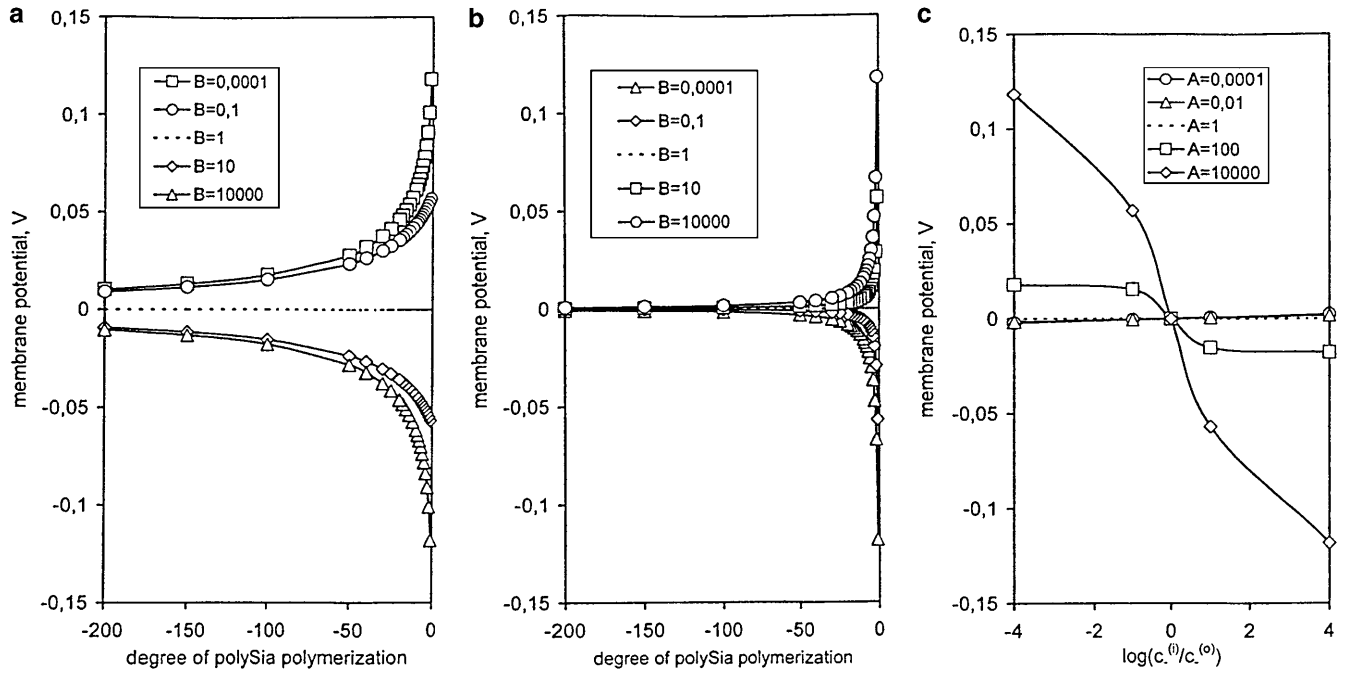

Fig. 6 **a** The transmembrane potential, V_m, as a function of the degree of polysialic acid (polySia) polymerization (presented in negative values which correspond to the polySia ionic valency, z_-). Each line was plotted for the constant ratio of polySia concentration, $B = c_-^{(i)}/c_-^{(o)}$. Results calculated for the ratio of the permeability coefficients P_+/P_- equal to 10^2 and a temperature of 298 K. **b** The transmembrane potential, V_m, as a function of the degree of polysialic acid (polySia) polymerization (presented in negative values which correspond to the polySia ionic valency, z_-). Each line was plotted for the constant ratio of polySia concentration, $B = c_-^{(i)}/c_-^{(o)}$. Results calculated for the ratio of the permeability coefficients P_+/P_- equal to 10^{-2} and a temperature of 298 K. **c** The transmembrane potential, V_m, as a function of the ratio of polysialic acid (polySia) concentrations, $c_-^{(i)}/c_-^{(o)}$. Each line was plotted for the constant ratio of the permeability coefficient, $A = P_+/P_-$. Results calculated for the polySia ionic valency equal to –100 and a temperature of 298 K

cationic species present in the bulk solution. This in turn reduces the surface potential. Based on the permeability properties for K^+, Na^+, and Ca^{2+} ions, the author suggested that hemolymph composition has evolved in such a way as to preserve the transmembrane potential across particular areas of the cell membrane. However, the existence of polyvalent ions, such as polysialic acid, has not been taken into account.

Equations have been derived, based on the Goldman-Hodgkin-Katz equation, for the transport of a symmetrical electrolyte, consisting of cations and anions of equal valency, through a neutral membrane that separates two solutions of finite volume under quasi-steady-state conditions (Ohshima et al. 1988). The authors proposed some simple approximate expressions for the solute concentrations and for the membrane potential as functions of time. Deviation of the time course of the solute concentrations from that of the neutral solutes was found to be determined by the permeability ratio of the cations and anions. In our work a more general case is considered in which the valencies of the cations and anions are not equal.

The voltage-sensitive sodium channel from eel electroplax is formed of a polypeptide, to which polysialic acid chains of estimated $DP = 113 \pm 11$ are attached (James and Agnew 1989). The authors indicated that these chains may serve to maintain a solute reservoir, creating an anionic cloud over the molecule, and the chains may shield the channel from extracellular proteins. It appears from our data that the appearance of polySia, containing over 100 negative charges, close to the sodium channel, can locally change the transmembrane potential, thus modulating the activity of this voltage-dependent channel. Similar phenomena can arise in the cell membrane of developing neurons, where the appearance, at the extracellular site, of polySia, attached to the NCAM integral protein, can locally modulate the membrane potential, which in turn can regulate the activity of membrane-bound enzymes, channels, and receptors (Wojtczak and Nałęcz 1985).

The inner membrane-associated polysialyltransferase complex in *E. coli* K1 catalyzes the synthesis, transmembrane translocation, and assembly of polySia (Troy 1992). The functional domain of the polysialyltransferase is localized on the cytoplasmic surface of the inner membrane and polySia chains are fully polymerized inside the cell before being translocated (Troy et al. 1990a, b). Both the proton transmembrane electrochemical potential gradient and the transmembrane electrical potential are actively involved in the vectorial translocation of polySia across the inner membrane (Troy et al. 1990b, 1991). It was postulated (Troy et al. 1991) that the relatively large transmembrane potential, up to –150 mV (negative inside), that is developed across the *E. coli* inner membrane may facilitate the movement of these polyanions across the membrane. Undecaprenyl

phosphate (C_{55}-P) is involved in the formation of the polysialic acid capsule in *E. coli* K1 bacterial cells (Troy 1979), and the interaction of the transmembrane potential gradient with the negative charge of the phosphate group of C_{55}-P can determine the dynamics, conformation, and aggregation behavior of C_{55}-P in membranes (Janas and Tien 1988; Janas et al. 1990; Janas and Janas 1995; Janas et al. 2000). It seems from the present study that the membrane potential generated by polySia translocation can superimpose the inner membrane potential, thus modulating the rate of polySia translocation and the dynamics of C_{55}-P molecules in the inner bacterial membranes.

Investigations of a wide range of human tumors indicate that many neuroendocrine tumors express polysialylated NCAM (Troy 1992; Rougon 1993). The involvement of the electric transmembrane potential in a contact inhibition of cell division has been reported (Cone and Tongier 1973) and the membrane potential theory of carcinogenesis has been presented (Beech 1989). We postulate that the involvement of polySia in the enhancement of the metastatic potential of cells and the invasive potential of tumors can arise from its ability to modulate the transmembrane potential gradient.

In conclusion, we have shown that the expression of polySia in bacterial cells through the transmembrane translocation process can be responsible for modulation of the transmembrane potential of the bacterial inner membrane. We suggest that the polySia chains can influence the transmembrane potential of neural cell membranes in a similar way. This analysis can also describe the effect of translocation of negatively charge polyanionic polynucleotydes (Xie et al. 1990) across the cell membrane on the cell membrane potential. Although the high concentration and membrane permeabilities for potassium and chloride ions can possibly significantly modulate the effect of the polyanions, the physiological importance of the presented data results from the ability of these polyanions to create local changes in the transmembrane potential at the translocation sites.

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