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Kinetically Different Populations of O-Pyromellityl-Gramicidin Channels Induced by Poly-L-Lysines in Lipid Bilayers

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Abstract. Clustering of membrane proteins, in particular of ion channels, plays an important role in their functioning. To further elucidate the mechanism of such ion channel activity regulation, we performed experiments with a model system comprising the negatively-charged gramicidin analog, O-pyromellitylgramicidin (OPg) that forms ion channels in bilayer lipid membrane (BLM), and polycations. The effect of polylysines on the kinetics of OPg channels in BLM was studied by the method of sensitized photoinactivation. As found in our previous work, the interaction of polylysine with OPg led to the deceleration of the OPg photoinactivation kinetics, i.e., to the increase in the characteristic time of OPg photoinactivation. It was shown here that in a certain range of polylysine concentrations the photoinactivation kinetics displayed systematic deviations from a monoexponential curve and was well described by a sum of two exponentials. The deviations from the monoexponential approximation were more pronounced with polylysines having a lower degree of polymerization. These deviations increased also upon the elevation of the ionic strength of the bathing solution and the addition of calcium ions. A theoretical model is presented that relates the OPg photoinactivation kinetics at different concentration ratios of OPg and polylysine to the distribution of OPg molecules among OPg-polylysine clusters of different stoichiometry. This model is shown to explain qualitatively the experimental results, although the quantitative description of the whole body of evidence requires further development, assuming that the interaction of polylysine with OPg causes segregation of membrane domains enriched in OPg channels. The single-channel data, which revealed the insensitivity of the single-channel lifetime of OPg to the addition of polylysine, are in good agreement with the theoretical model.

Key words: Polylysine — Sensitized photoinactivation — Gramicidin — Ion channel — Bilayer lipid membrane — Membrane domain

Introduction

Recently the concept of the existence of lipid rafts differing in lipid and protein composition from the surrounding bulk of biological membranes has been put forward and a variety of data favoring this concept have been obtained (for review *see* Brown & London, 2000). It is supposed that the processes of raft formation and functioning, in particular those associated with signal transduction, involve clustering of membrane-bound and integral proteins that is governed by specific receptor-ligand interactions (Bormann & Engelman, 1992; Metzger, 1992; Lemmon & Schlessinger, 1994; Simons & Toomre, 2000; Babiychuk & Draeger, 2000; Bouvier, 2001; Cochran et al., 2001). However, the contribution of nonspecific electrostatic interactions to the raft dynamics cannot be excluded. It has been argued theoretically that the formation of lipid-protein domains in membranes may be promoted by electrostatic adsorption of proteins (Denisov et al., 1998; Heimburg, Angerstein & Marsh, 1999; May, Harries & Ben-Shaul, 2000). Our data on the interaction of polyelectrolytes (polylysines and Konig's polyanion) with negatively

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Abbreviations: BLM, bilayer lipid membrane; DPhPC, diphytanoylphosphatidylcholine; PLL, poly-L-lysine; OPg, O-pyromellitylgramicidin

and positively charged gramicidins incorporated into a lipid membrane (Krylov et al., 1998, 2000; Antonenko et al., 2002) have shown the possibility of studying segregation of membrane proteins into domains by using a simple model system (Rokitskaya, Antonenko & Kotova, 1996). The results presented in our previous papers (Krylov et al., 1998, 2000) have demonstrated that electrostatic interactions belong to the critical factors driving the process of integral protein clustering in model membranes. The peculiar feature revealed by our data consists of the fact that the process of lateral segregation of charged membrane peptides displays an optimum at a certain concentration of an oppositely charged polyelectrolyte that binds to the membrane and induces the domain formation (Krylov et al., 2000). This result is in line with the data on the effects of polycations and polyanions on distribution of charged lipids in bilayers (Junker & Creutz, 1993; Mitrakos & Macdonald, 1997; Raudino & Casteli, 1997; Hinderliter et al., 1998, 2001; Hirsch-Lerner & Barenholz, 1998; Macdonald et al., 1998; Subramanian et al., 2000; Franzin & Macdonald, 2001). The present paper focuses on the careful analysis of the kinetics of the photoinactivation of O-pyromellitylgramicidin (OPg, a gramicidin analog that has three negative charges at the C-terminus) channels in planar bilayer lipid membranes (BLM) in the presence of polylysine. We show here that the time course of the photosensitizer-mediated decrease in the electric current through OPg channels after a single flash of visible light, i.e., the OPg photoinactivation kinetics that reflects the kinetic characteristics of the gramicidin channels (Rokitskaya et al., 1996), is rather complicated at the intermediate concentrations of polylysine. Actually, the photoinactivation kinetics, being well-described by single exponentials both at low and high polylysine concentrations, exhibited substantial deviations from a monoexponential curve at the intermediate polylysine concentrations. The data are discussed in terms of the concept of heterogeneity of OPg population in BLM, attributed to the formation of ensembles of OPg channels interacting with polylysine that differ from free OPg channels in their kinetic properties.

Materials and Methods

BLMs were formed from a 2% solution of diphytanoylphosphatidylcholine (DPhPC, Avanti Polar Lipids, Alabaster, AL) in *n*-decane (Merck, Darmstadt, Germany) by the brush technique (Mueller et al., 1963) on a 0.55-mm diameter hole in a Teflon partition separating two compartments of a cell. Each compartment contained an aqueous solution of 100 mM (unless otherwise stated) KCl (Fluka, Buchs, Switzerland), 10 mM MES (Sigma, St.Louis, MO), 10 mM TRIS (Sigma, St.Louis, MO) and 0.1 mM EDTA at pH 7.0. O-pyromellitylgramicidin (OPg) chemically synthesized as described by Cifu, Koeppe & Andersen (1992) (it was a generous gift of Prof. R.E. Koeppe II, University of Arkansas) was added from stock solutions in ethanol to the bathing solutions at both sides of the BLM and routinely incubated for 15 min with constant stirring. Poly-L-lysine hydrobromides with molecular weights equal to 3,800 (degree of polymerization (DP) = 18, PLL_{18}); 7,500 (DP = 35, PLL_{35}); 12,000 (DP = 60, PLL_{60}), (Sigma, St.Louis, MO) were added to both compartments of the cell. Polylysine chains were always in great excess with respect to OPg. In fact, the concentration of OPg in the bathing solution varied from 0.5 to 2 nM, whereas the effective concentration of PLL was of the order of 1 μM. Taking into account that only a small part of OPg was incorporated in the BLM, the ratio of the number of PLL chains to that of the membrane-bound OPg molecules was even higher. Experiments were carried out at room temperature (20–22°C). Aluminum trisulfophthalocyanine ($AlPcS_3$) was from Porphyrine Products, Logan, UT. $AlPcS_3$ was added to the bathing solution at the trans-side (the cis-side is the side facing the flash lamp). The electric current (I) was recorded under voltage-clamp conditions. The currents were measured by a U5-11 amplifier (Moscow, Russia), digitized by a LabPC 1200 (National Instruments, Austin, TX) and analyzed using a personal computer with the help of WinWCP Strathclyde Electrophysiology Software designed by J. Dempster (University of Strathclyde, UK). The voltage of 30 mV was applied to BLM with Ag-AgCl electrodes placed directly into the cell. BLMs were illuminated by single flashes produced by a xenon lamp with a flash energy of about 400 mJ/cm^2 and a flash duration <2 msec. To avoid electrical artifacts, the electrodes were covered by black plastic tubes.

In the presence of a photosensitizer (e.g., aluminum phthalocyanine or Rose Bengal, dyes that sensitize singlet oxygen formation with high quantum yield), irradiation of BLM with visible light is known to decrease the gramicidin-mediated transmembrane current, I (Strassle & Stark, 1992; Rokitskaya et al., 1993). The decrease in the current is believed to result from damage to tryptophan residues of gramicidin (tryptophan oxidation and/or peptide fragmentation) (Strassle & Stark, 1992; Kunz et al., 1995) caused by reactive oxygen species that are generated upon excitation of a photosensitizer (Rokitskaya et al., 1996, 2000). If BLM is illuminated with a single flash of visible light, I is a monoexponential function of time (Rokitskaya et al., 1996): $I(t) = (I_0 - I)\exp(-t/\tau) + I_\infty$, where I_0, I_∞ and τ are the initial current prior to illumination, the stationary level of the current established as a result of relaxation after the flash, and the characteristic time of photoinactivation, respectively. The relative amplitude of photoinactivation, α, is defined as $\alpha = (I_0 - I_\infty)/I_0$. Because the light-induced decrease in the gramicidin-mediated current is due to the reduction of the number of open channels (Rokitskaya et al., 1993; Kunz et al., 1995), α is equal to the damaged part of gramicidin channels.

In single-channel experiments, the voltage of 100 mV was applied to BLM and currents were measured by an OES-2 patch-clamp amplifier (Opus, Moscow). WinEDR software was used for the current recordings and analysis (Strathclyde Electrophysiology Software designed by J. Dempster, University of Strathclyde, UK).

Results

Theoretical Part

Let us consider a system comprising positively charged polylysine (PLL) molecules floating in the bathing solution and those adsorbed on BLM, on one hand, and negatively charged O-pyromellitylgramicidin (OPg) molecules incorporated in BLM, on the other hand

(Fig. 1). Let's assume that both the total concentration of OPg in BLM and the concentration of PLL chains in the aqueous solution are constant and equal to N and [PLL], respectively. The latter condition means that the bathing solution represents a polylysine stock of infinite capacity, which is valid for planar BLMs (and normally invalid for liposomes). The interaction of polylysine with gramicidin at one side of the membrane can be described by a system of reactions:

$$\begin{aligned} &\mathrm{PLL} + \mathrm{gram} \Leftrightarrow \mathrm{PLL}_1 \\ &\mathrm{PLL}_1 + \mathrm{gram} \Leftrightarrow \mathrm{PLL}_2 \qquad (1) \\ &- - - - - - - - - - - - - - \\ &\mathrm{PLL}_{v-1} + \mathrm{gram} \Leftrightarrow \mathrm{PLL}_v \end{aligned}$$

where v is the maximum number of binding sites belonging to a single polylysine chain; $\mathrm{PLL_n}$ is the concentration of polylysine chains, each bound to n OPg molecules incorporated in BLM, with n ranging from 1 to v and gram is the concentration of free (not bound to polylysine) OPg molecules. The top equation in (1) is characterized by a three-dimensional binding constant K_1 (in M^{-1}), while the other equations are characterized by two-dimensional binding constants K_X (in cm^2/mole). For the purpose of simplicity, let us assume that the K_X values in all reactions of the system are identical (actually, K_X may depend on the portion of monomer units of a polyelectrolyte involved in binding (Polinsky, Pshezhetsky & Kabanov, 1981)). Because the total concentration of OPg is equal to N, it can be written:

$$\begin{aligned} &[\mathrm{gram}] + [\mathrm{PLL}_1] + 2[\mathrm{PLL}_2] + \cdots + v[\mathrm{PLL}_v] \\ &\quad = [\mathrm{gram}] + \sum_{n=1}^{v} n[\mathrm{PLL_n}] = N \qquad (2) \end{aligned}$$

According to Perelson (1981), Kim et al. (1991), Stone, Cochran & Stern (2001), the above system of equations can be converted to the following equation:

$$N = [\mathrm{gram}] + \sum_{n=1}^{v} n \frac{v!}{n!(v-n)!} K_1[\mathrm{PLL}](K_X[\mathrm{gram}])^{v-1}[\mathrm{gram}]$$

By using dimensionless parameters, the last equation can be rewritten as follows:

$$x + \sum_{n=1} n \frac{v!}{n!(v-n)!} cx^n = x(1 + vc(1+x)^{v-1}) = b \qquad (3)$$

where $x = [\mathrm{gram}]K_X$, $c = [\mathrm{PLL}]K_1$ and $b = NK_X$.

This equation was solved numerically. The solution enables us to calculate the distribution of PLL chains bound to different numbers of OPg molecules.

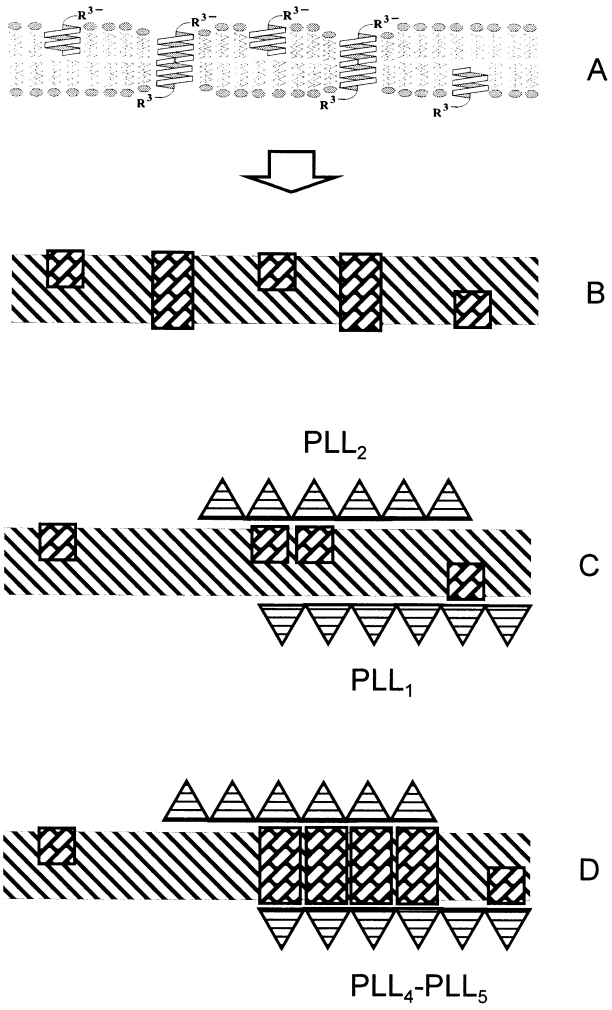

Fig. 1. Scheme of interaction of negatively charged gramicidin (O-pyromellitylgramicidin, OPg) with polylysine at the membrane surface. OPg monomers and OPg channels (dimers) are drawn as rectangles of different sizes (compare panels *A* and *B*), and polylysine chains, as chains of triangles (panel *C*). $\mathrm{PLL_2}$ in panel *C* is a complex (cluster) of two OPg monomers with a PLL chain (the chain presented has maximum capacity (v) to bind 6 OPg monomers). $\mathrm{PLL_4}$-$\mathrm{PLL_5}$ is a transmembrane cluster of 4 channels (OPg dimers) and one OPg monomer.

Below, we present the results of calculations at $v = 10$ (Fig. 2). It has occurred that at $b < 1$ (i.e., at low concentrations of OPg in BLM) an increase in the PLL concentration never leads to the appearance of chains with $n > 1$ (black bars): at low values of c, PLL chains do not bind OPg at all ($n = 0$ in panels *A* and *B* of Fig. 2), whereas at high values of c, the population of PLL chains in which each chain is bound to one OPg molecule ($n = 1$, panel *D* of Fig. 2), predominates. The distribution of OPg is quantitated in $w_n = n[\mathrm{PLL_n}]/N$, ($w_0 = [\mathrm{gram}]/N$), so that the sum of all w_n is equal to unity (*see* Eq. 2).

At high concentrations of OPg in BLM (for example, at $b = 10$) the pattern of the distribution is more complicated. Under these conditions, free PLL

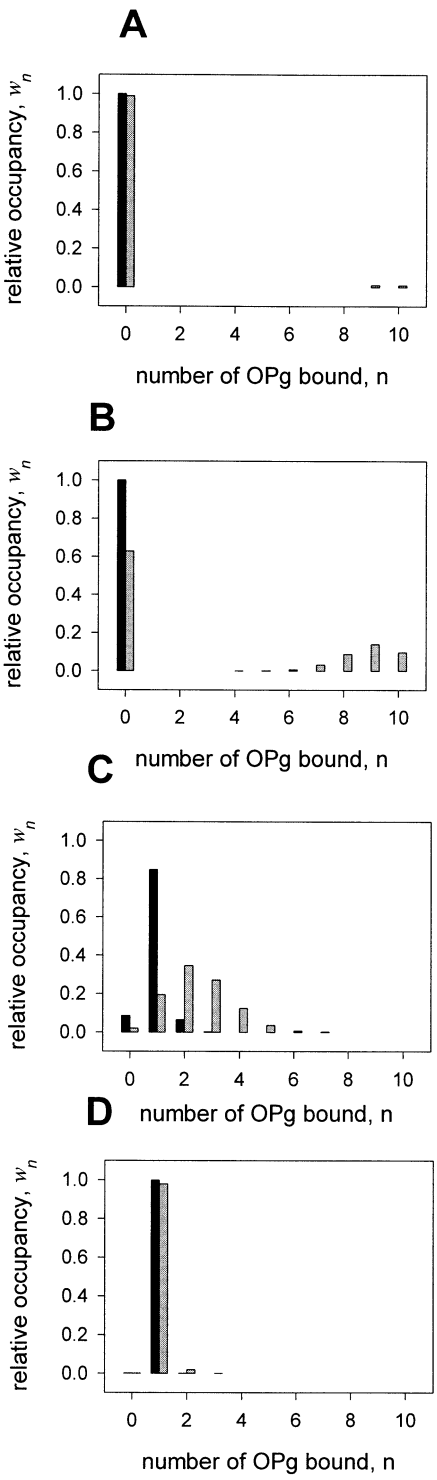

Fig. 2. Theoretical dependence of the distribution of the cluster composition on the concentration of polylysine at different OPg concentrations. Calculations of the relative occupancy of polylysine chains by OPg monomers were carried out according to Eq. 3, assuming $v = 10$. The value of c was 10^{-12}, 10^{-9}, 1 and 10^3. The value of b was 0.1 (*black columns*) and 10 (*shadowed columns*), respectively.

chains ($n = 0$) completely predominate only at very low values of c (Fig. 2*A*, shadowed bars). Beginning from a certain value of c, PLL chains with $n = 10$ appear to contribute substantially to the distribution (Fig. 2*B*), i.e., even at rather low PLL concentration, a noticeable population of PLL chains has got all binding sites occupied, and there is a trough in the distribution in the range of intermediate values of n. This trough is associated with a high value of b ($NK_x \gg 1$), meaning that the surface concentration of OPg is so high that once a PLL chain has bound to a single OPg molecule at the membrane surface, other OPg molecules fill all the binding sites on the polymer chain. As c is increased further, the distribution of PLL chains with different values of n becomes more regular, that is, the contribution of chains having $1 < n < 10$ increases (Fig. 2*C*). This change in the distribution occurring at higher PLL concentrations, i.e., a decrease in the number of fully occupied polymer chains and the appearance of PLL chains with intermediate n values, apparently results from the competition between a high number of bound polymer chains for a limited number of OPg molecules incorporated in the membrane. Ultimately, at high values of c, the distribution displays a sharp peak at $n = 1$. Though surprising at first glance, the pattern seen in this limiting case is readily explained. Actually, under conditions of great excess of PLL over OPg, the PLL competition for a limited number of binding sites (OPg) on the membrane leads to the situation that each bound polymer chain carries only one OPg molecule (Fig. 2*D*).

Thus, the model described allows one to find the ranges of the parameter values that characterize various degrees of OPg clustering in a single monolayer of BLM. Bearing in mind that gramicidin channels represent transmembrane dimers (Andersen et al., 1999), aggregation of OPg monomers induced by interaction with PLL may lead to formation of transmembrane clusters like PLL_{n1}-PLL_{n2} (structure in panel *D*, Fig. 1), along with more sophisticated complexes. In particular, transmembrane association of clusters may result in segregation of gramicidin domains in a lipid membrane.

The precise analysis of this system is extremely complicated. However, at first approximation we can suggest that a probability of the existence of a transmembrane PLL_{n1}-PLL_{n2} cluster ($w_{n1,n2}$) is the product of probabilities of formation of PLL_{n1} cluster in a monolayer 1 (w_{n1}) and that of PLL_{n2} cluster in monolayer 2 (w_{n2}), multiplied by the lifetime of the transmembrane cluster.

$$w_{n1,n2} = w_{n1} w_{n2} \tau_{n1,n2} \tag{4}$$

Let us assume that the lifetime of the cluster increases proportionally with the number of transmembrane dimers in this cluster:

$$\tau_{n1,n2} = \min\{n1, n2\}(\tau_{max} - \tau_0)/v \tag{5}$$

where τ_0 and τ_{max} are the values of OPg channel lifetime in the absence of PLL, and the lifetime of the PLL_v-PLL_v cluster, respectively.

Based on consideration performed in our previous papers (Rokitskaya et al., 1996; Antonenko et al., 2002), it is possible to relate the lifetime of the OPg channel cluster to the experimental parameter — the characteristic time of gramicidin photoinactivation defined in Materials and Methods. The equilibrium between gramicidin monomers (A) and dimers (A_2) in a membrane depends on the reaction rate constants k_R and k_D as follows:

$$A + A \underset{k_D}{\overset{k_R}{\Leftrightarrow}} A_2 \tag{6}$$

According to Rokitskaya et al. (1996), the flash-induced decay of the gramicidin-mediated current in the presence of the photosensitizer, here called the kinetics of photoinactivation, represents the relaxation process after a concentration jump, i.e., the gramicidin monomer-dimer equilibration. As shown by Rokitskaya et al. (1996), and Antonenko et al. (2002), the characteristic time of photoinactivation (τ) is determined by the rate constants of formation (k_R) and dissociation (k_D) of gramicidin dimers, according to the following equation:

$$1/\tau = k_D + 4k_R N_{1\infty} \tag{7}$$

where $N_{1\infty}$ is the concentration of gramicidin monomers in BLM at the equilibrium. Because the value of the single-channel lifetime of OPg, which is equal to $1/k_D$ (Bamberg & Läuger, 1973), is close to the characteristic time of photoinactivation (0.4 sec and 0.3 sec, respectively) (Krylov, 2000), it can be concluded that under our experimental conditions $k_R \times N_{1\infty} \ll k_D$, i.e., the characteristic time of photoinactivation is reciprocal to k_D.

The overall photoinactivation kinetic curve at given values of c and b (Eq. 3) can be calculated by summation of the exponential decay curves related to each cluster with corresponding weights estimated according to the distribution described above:

$$I(t) = \sum_{n1,n2}^{v} w_{n1,n2} \exp(t/\tau_{n1,n2}) \tag{8}$$

Figure 3 presents theoretical curves calculated for the parameter values that were used in the analysis of the distribution of PLL chains in Fig. 2. Each theoretical curve (crosses in panels *A–D*) was approximated by a monoexponential function (*solid curves* in panels *A–D*) to estimate the magnitude of possible deviations. As seen from Fig. 3, the substantial deviations from a single exponential occur only at $c = 10^{-9}$ (panel *B*). In general, the overall kinetic curve changes from the fast monoexponential decay in the absence of PLL to the biexponential curve at $c = 10^{-9}$, then to slow monoexponential kinetics at $c = 1$ and finally to fast monoexponential kinetics at $c = 10^3$.

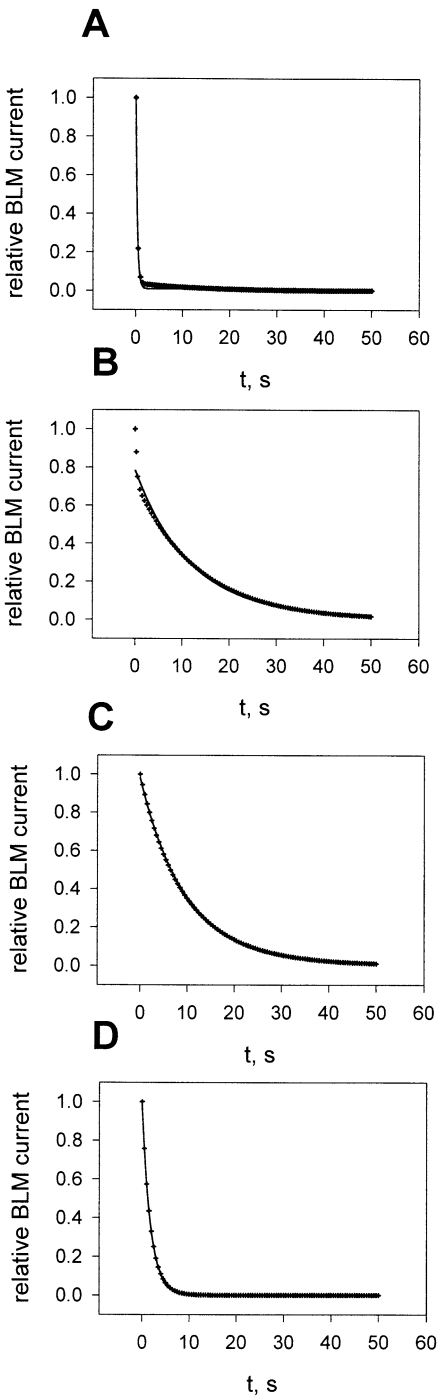

Fig. 3. Theoretical dependence of the photoinactivation kinetics on the concentration of polylysine at a high OPg concentration ($b = 10$). Calculations of the kinetic curves (crosses) were carried out according to Eqs. 7 and 8, assuming $\tau_0 = 0.3$ sec and $\tau_{max} = 15$ sec. Other parameters were the same as in Fig. 2. Solid curves represent the best fit of the theoretical kinetics with single exponentials having characteristic times of 0.33 sec (panel *A*), 11.7 sec (*B*), 9.6 sec (*C*), and 1.8 sec (*D*), respectively.

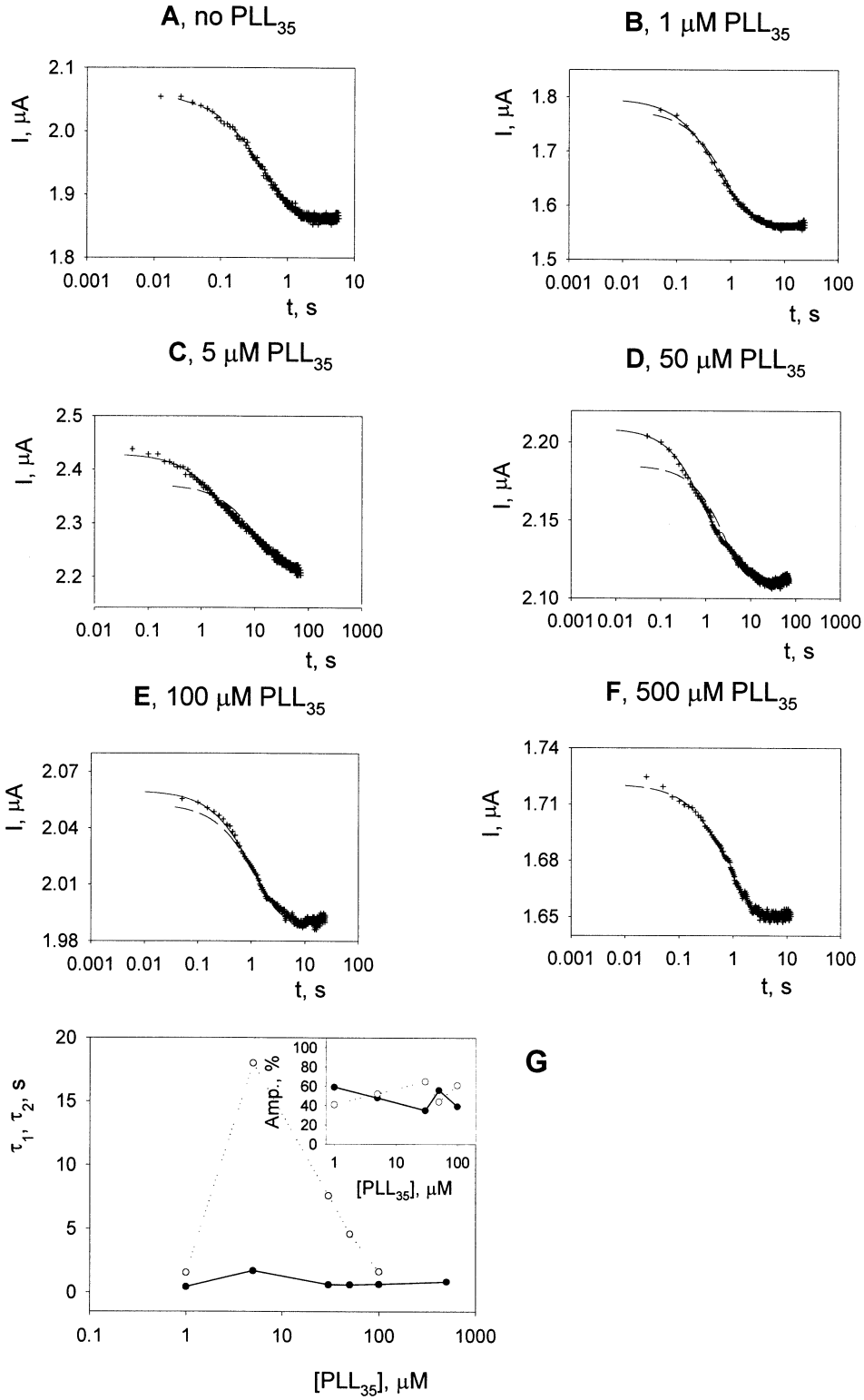

Fig. 4. The kinetics of the decrease in the O-pyromellitylgramicidin-mediated current (*I*) through BLM in the presence of 1 μM $AlPcS_3$ after a flash of visible light (at zero time) at different concentrations of PLL_{35} (panels *A–F*) and the concentration dependence of two characteristic times (τ_1 and τ_2, panel *G*). The experimental results were fitted with single exponentials (*dashed curves*) and double exponentials (*solid curves*) with characteristic times (and relative amplitudes, *see* inset) plotted in panel *G*. The solution contained 100 mM KCl, 10 mM MES, 10 mM TRIS and 0.1 mM EDTA, pH 7.0.

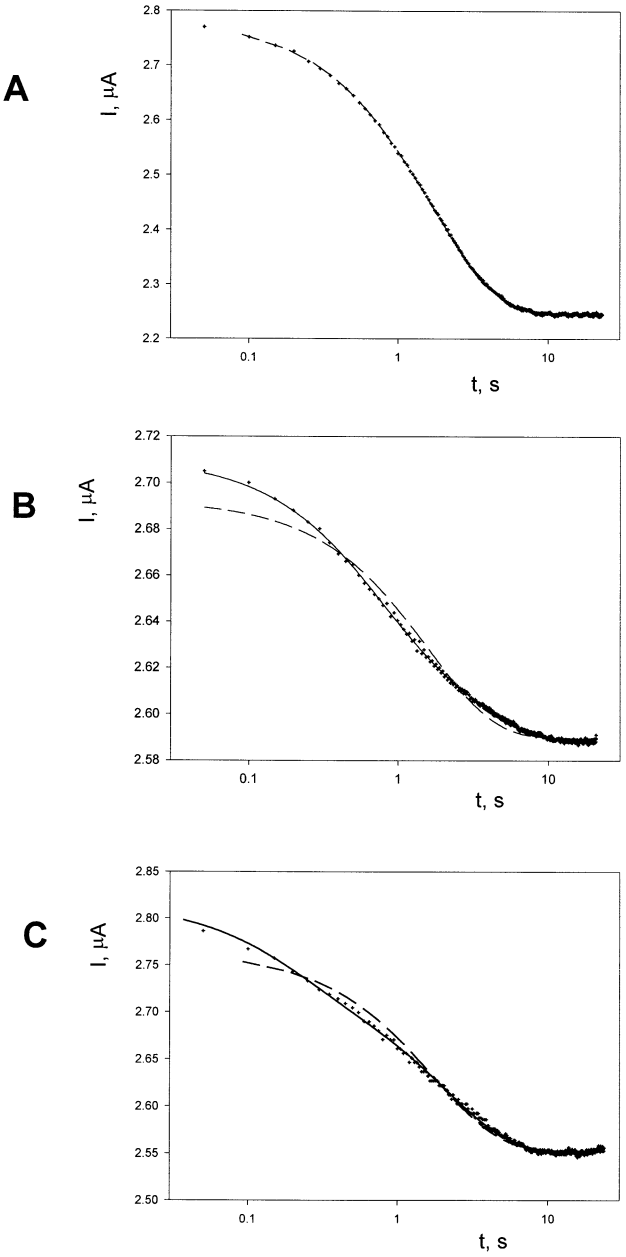

Fig. 5. The kinetics of the decrease in the O-pyromellitylgramicidin-mediated current (I) through BLM in the presence of 1 μM $AlPcS_3$ after a flash of visible light (at zero time) in the presence of 3 μM PLL_{60}, (panel A), 5 μM PLL_{35} (panel B), and 20 μM PLL_{18} (panel C). The experimental data points were fitted with single exponentials (dashed curves) with characteristic times $\tau = 1.69$ sec (A), $\tau = 1.62$ sec (B), $\tau = 1.77$ sec (C). In the case of PLL_{35} and PLL_{16}, double-exponential fits $I = I_0 + \alpha_1\exp(-t/\tau_1) + \alpha_2\exp(-t/\tau_2)$ (*solid curves*) were also calculated with the following parameters: $\alpha_1/(\alpha_1+\alpha_2) = 45\%$, $\tau_1 = 0.62$ sec, $\alpha_2/(\alpha_1+\alpha_2) = 55\%$, $\tau_2 = 2.90$ sec (B), and $\alpha_1/(\alpha_1+\alpha_2) = 33\%$, $\tau_1 = 0.19$ sec, $\alpha_2/(\alpha_1+\alpha_2) = 67\%$, $\tau_2 = 2.08$ sec (C). The control τ (in the absence of PLL) was 0.3 sec. The solution contained 100 mM KCl, 10 mM MES, 10 mM TRIS and 0.1 mM EDTA, pH 7.0.

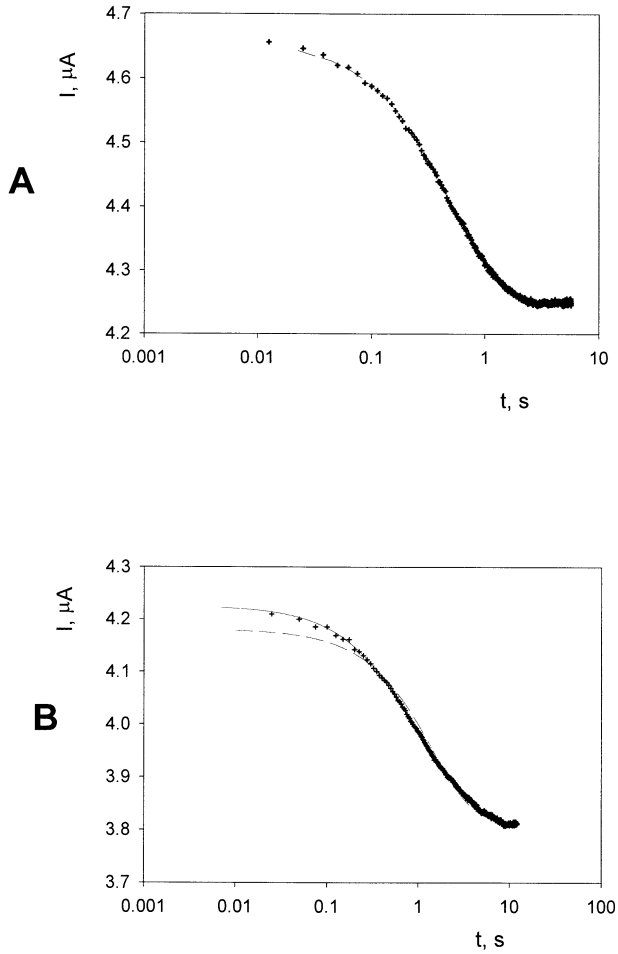

Fig. 6. The kinetics of the decrease in the O-pyromellitylgramicidin-mediated current (I) through BLM in the presence of 1 μM $AlPcS_3$ after a flash of visible light (at zero time) in the absence of PLL_{60} (panel A) and in the presence of 5 μM PLL_{60} (panel B). The solution contained 200 μM KCl. The experimental data points were fitted with single exponentials (*dashed curves*) with characteristic times $\tau = 0.52$ sec (A), and $\tau = 1.46$ sec (B). In the presence of PLL_{60}, double-exponential fits $I = I_0 + \alpha_1\exp(-t/\tau_1) + \alpha_2\exp(-t/\tau_2)$ (*solid curves*) were also calculated with the following parameters: $\alpha_1/(\alpha_1+\alpha_2) = 49\%$, $\tau_1 = 0.57$ sec, $\alpha_2/(\alpha_1+\alpha_2) = 51\%$, $\tau_2 = 2.50$ sec (B).

Basically, these kinetic features reflect the distributions of PLL_n chains shown in Fig. 2. In fact, the model suggests that the increase in τ is related to the appearance of OPg-PLL complexes of high n values (Eq. 5). According to Fig. 2, these complexes are formed only at intermediate concentrations of PLL due to the competition of PLL chains for a limited number of OPg molecules in the membrane. In the limiting case of very high PLL concentrations, the complexes, in which a PLL chain is bound to only one OPg molecule (PLL_1), predominate. These complexes are supposed to form transmembrane dimers of the PLL_1-PLL_1 structure characterized by τ close to that of OPg channels without polylysine.

Experimental Part

Figure 4 displays the time courses of the decrease in OPg-mediated current across BLM (below called the

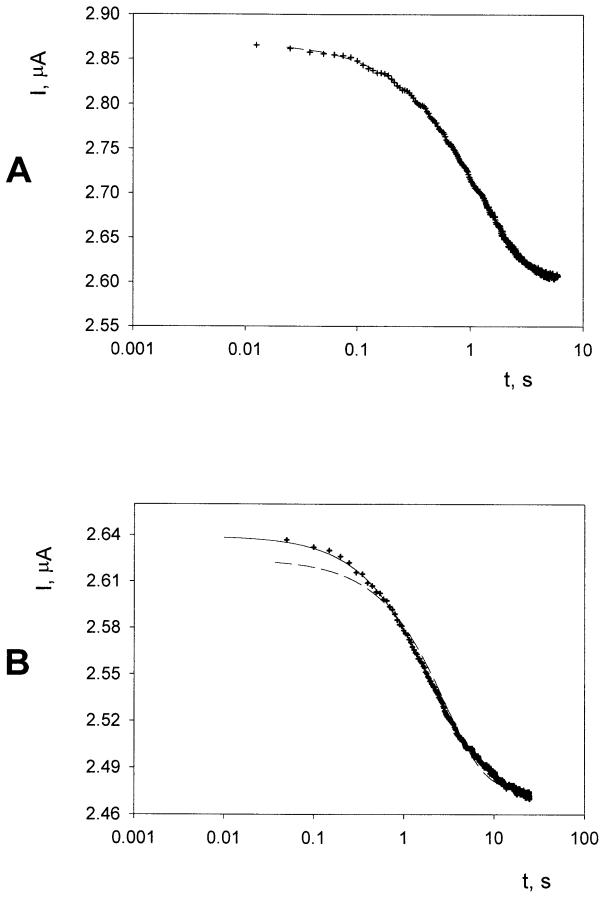

Fig. 7. The kinetics of the decrease in the O-pyromellitylgramicidin-mediated current (I) through BLM in the presence of 1 μM $AlPcS_3$ after a flash of visible light (at zero time) in the absence of PLL_{60} (panel *A*) and in the presence of 5 μM PLL_{60} (panel *B*). The solution contained 3 mM $CaCl_2$. The experimental data points were fitted with single exponentials (*dashed curves*) with characteristic times $\tau = 1.18$ sec (*A*), and $\tau = 2.93$ sec (*B*). In the presence of PLL_{60}, double-exponential fits $I = I_0 + \alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2)$ (*solid curves*) were also calculated with the following parameters: $\alpha_1/(\alpha_1+\alpha_2) = 69\%$, $\tau_1 = 1.52$ sec $\alpha_2/(\alpha_1+\alpha_2) = 31\%$, $\tau_2 = 7.31$ sec (*B*).

kinetics of OPg photoinactivation) obtained at different concentrations of PLL_{35}. In agreement with previous results (Krylov et al., 1998, 2000), the control kinetics measured in the absence of polylysines was strictly monoexponential with τ of 0.45 sec. It is seen that within the range of intermediate polylysine concentrations (panels *C* and *D*), which caused maximal deceleration of the photoinactivation kinetics, there were substantial deviations from monoexponential curves. On the other hand, the experimental kinetic curves were well approximated by theoretical curves representing a sum of two exponentials (*solid curves*). Panel *G* shows that the exponential factor of one of these two components (τ_1) changed considerably upon variation of the PLL_{35} concentration, while the contributions of two exponential components to the overall kinetics were almost independent of the polylysine concentration (Fig. 4*G*, insert).

Figure 5 demonstrates a series of the kinetic curves obtained in the presence of polylysines of different degrees of polymerization (60, 35 and 18). It is seen that with 3 μM PLL_{60}, the time course of the BLM current was fitted well by a monoexponential curve with $\tau = 1.7$ sec (panel *A*), whereas with PLL_{35} and PLL_{18} the fitting revealed noticeable deviations from a single exponential (panels *B*, *C*).

As shown previously (Krylov et al., 2000), the increase in the ionic strength leads to the reduction of the polylysine effect on the OPg channel kinetics, in particular, to a marked decrease in the maximal value of τ. It is seen from Fig. 6 that the deviations from the monoexponential kinetics were also observed with PLL_{60} upon elevation of the ionic strength of the bathing solution from 100 to 200 mM KCl. A similar effect was produced by calcium ions (Fig. 7).

To probe the interaction of polylysine with OPg at the single-channel level, we performed single-channel experiments. Figure 8 shows the single-channel recordings, current histograms and channel-duration histograms of OPg in the absence (*left row*) and in the presence of 15 μM PLL_{35} (*right row*). The addition of PLL_{35} did not produce any effect on the single-channel lifetime, which was equal to 0.42 sec. By contrast, in the multi-channel experiments (with the current of about 1 μA at 30 mV) the addition of 15 μM PLL_{35} led to the increase in the characteristic time of photoinactivation from 0.3 sec to 10 sec (*data not shown*).

Discussion

The model presented in the theoretical part of this paper considers the formation of clusters of the negatively charged gramicidin analog, OPg, resulting from its interaction with polylysine, and estimates the composition of the clusters upon varying both the concentration of OPg in BLM and the concentration of polylysine chains in the bathing solution. This model relates the characteristic time of photoinactivation (τ) to the lifetime of PLL-OPg clusters of different stoichiometry, and thereby explains qualitatively the effect of PLL on the OPg photoinactivation kinetics, in particular: 1) the biphasic (bell-shaped) dependence of τ on the PLL concentration (Fig. 4 and *see* also Krylov et al. (2000)); 2) the dependence of the PLL-induced increase in τ on the concentration of OPg (the effect is absent at the single-channel level (Fig. 8) and well pronounced at the multi-channel level (Fig. 4), *see* also Krylov et al. (1998) and 3) the biexponential character of the photoinactivation kinetics in the intermediate range of PLL concentrations observed here.

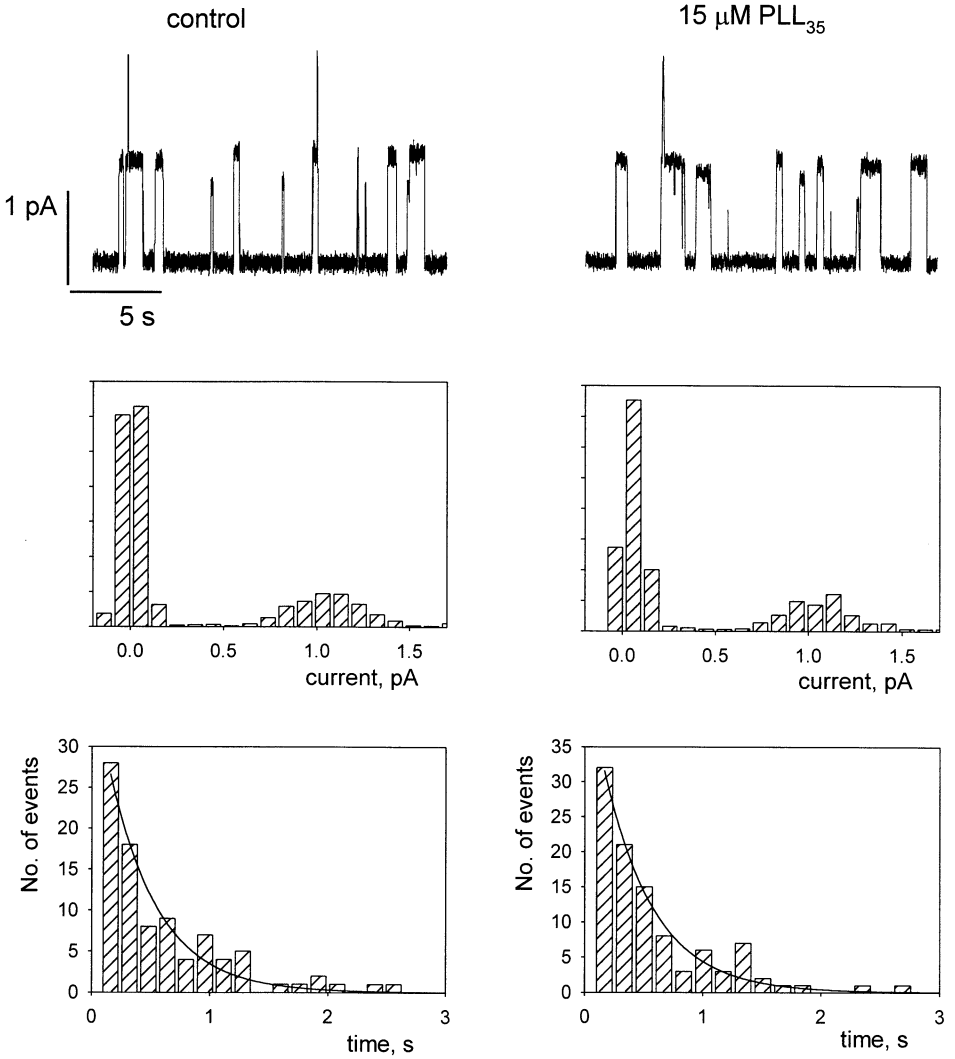

Fig. 8. Single-channel recordings (*top*), current histograms (*middle*), and channel-duration histograms (*bottom*) of OPg channels in DPhPC bilayers at 100 mV in the absence (*left side*) and in the presence of 15 μM PLL_{35} in the bathing solutions (*right side*). Current histograms were plotted from the 200-sec recordings. Duration histograms were fitted by single exponentials with an exponential factor of 0.42 sec in the control and 0.41 sec in the presence of PLL_{35} (*solid curves*). The solution contained 100 mM KCl, 10 mM MES, 10 mM TRIS and 0.1 mM EDTA, pH 7.0.

As mentioned above, the characteristic time of OPg photoinactivation displays the biphasic dependence on PLL concentration: as [PLL] rises, τ increases up to a maximum and starts to decrease when [PLL] exceeds a certain value (Krylov et al., 2000). This bell-shaped dependence is clearly predicted by the theoretical model (*see* Fig. 3). The decrease in τ at high PLL concentrations is accounted for by the disappearance of clusters of high n values due to the competition of PLL chains for a limited number of binding sites (OPg) in the membrane.

It has been shown earlier (Krylov et al., 1998) that the PLL effect on τ measured at the optimal concentration of PLL essentially depends on the magnitude of the electric conductance of BLM, i.e., on the OPg concentration: the increase in τ occurs only when the current exceeds a certain threshold value (equal to or higher than 10^{-7} A); then the effect grows sigmoidally with the current and reaches saturation at the current value corresponding to 10^{-6} A. This pattern of dependence is consistent with the theoretical model showing that the PLL effect on τ should develop at [OPg] $> K_X$, because the contribution of PLL chains having more than one binding site occupied by OPg is negligible at [OPg] $< K_X$ (Fig. 2). In line with this conclusion, the results of single-channel experiments (Fig. 8) clearly demonstrated the absence of the PLL effect at the single-channel level when [OPg] is extremely low.

Figure 4 shows that the addition of PLL of low degree of polymerization (PLL_{35}) led to the appearance of two kinetically different populations of OPg

channels, one of which had the value of τ close to the control one and the other was characterized by a much higher value of τ. In good agreement with the theoretical model, this heterogeneity manifested itself at intermediate PLL concentrations when OPg was involved partially in the interaction with PLL.

As seen from Figs. 6 and 7, the increase in the ionic strength and the addition of calcium ions to the bathing solution also led to the appearance of two populations of OPg. The kinetic heterogeneity was not observed in our earlier studies, because all the previous experiments were performed with PLL_{60} in the presence of 100 mM KCl, i.e., under conditions when the time course of the photoinactivation was well described by a monoexponential curve (*cf.* Fig. 5*A*).

It should be noted that the current theoretical model considering the formation of OPg-PLL clusters does not predict exactly the behavior of OPg photoinactivation kinetics observed in the experiments. For example, the model predicts that the range of the effective PLL concentrations should expand upon increasing the degree of polymerization, which contradicts with the experimental data (Krylov et al., 2000). Besides, the cluster model does not explain the preconditions for the appearance of the kinetic heterogeneity: a low length of a PLL chain and/or low ionic strength. We believe that these discrepancies can be overcome by further development of the theory, e.g., by taking into consideration the lateral segregation of OPg-PLL clusters, which leads to the appearance of densely packed domains of channels (the domain phase), along with freely diffusing OPg clusters and unbound channels. Moreover, the domain phase may be itself heterogeneous both in its composition and in the kinetic characteristics.

In terms of the domain hypothesis, the dependence of the kinetic pattern on the degree of PLL polymerization can be accounted for as follows: the addition of PLL_{60} at a certain concentration induces a highly cooperative demixing of OPg clusters from the free state in BLM to the domain phase, and it is practically impossible to observe the intermediary state when the photoinactivation kinetics displays the deviations from a monoexponential curve. It is known that all the above-mentioned factors (the increase in the ionic strength, Ca^{2+}, as well as the shortening of a polymer chain) lead to weakening of the electrostatic interaction between polyelectrolyte and membrane surface (Sixl & Galla, 1981; Carrier et al., 1985; Carrier & Pezolet, 1986; McElhaney, 1986; Laroche, Carrier & Pezolet, 1988; Heimburg & Marsh, 1995; Yang & Glaser, 1995; Ben-Tal et al., 1996, 1997; Heimburg et al., 1999; Murray et al., 1998, 1999; Wang et al., 2001; Kozlova et al., 2001), thereby reducing the degree of cooperativity of the demixing process. The data presented in Figs. 4–7 seem to be reasonably explained in terms of the domain hypothesis, because the efficiency of the interaction determines the degree of cooperativity of the transition from the state of free OPg clusters in BLM to the state of OPg channels joined into domains.

Nevertheless, clustering of OPg channels is the prerequisite for the formation of the domain phase in BLM and, therefore, the simple cluster model defines the conditions when the domain formation is possible. This is apparently the reason why the model presented can explain the main features of the experimentally observed properties of the system.

It can be concluded that the present work, based on our previous study (Krylov et al., 2000), provides a more precise analysis of the OPg channel kinetics in the presence of polylysine, which has revealed the conditions of the existence of OPg channels in states differing in their kinetic parameters. This model system allows us to gain insight into the prerequisites and consequences of the lateral distribution of charged molecules in membranes upon interaction with polycations. It is known that such interaction occurs in cellular membrane systems and can play an important role in certain physiological processes (Glaser et al., 1996; Murray et al., 1997; Mouritsen & Jorgensen, 1994, 1995, 1997).

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