Polylysine decelerates kinetics of negatively charged gramicidin channels as shown by sensitized photoinactivation

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Abstract Effect of a cationic polymer, poly(L-lysine), on the kinetic properties of ionic channels formed by neutral gramicidin A (gA) and its negatively charged analogue *O*-pyromellitylgramicidin (OPg) in a bilayer lipid membrane is studied using a method of sensitized photoinactivation. This newly developed method is based on the analysis of transmembrane current transients induced by a flash in the presence of a photosensitizer. It has been shown previously that the time course of the flash-induced current decrease in most cases follows a single exponential decay with an exponential factor (τ, the characteristic time of photoinactivation) that correlates well with the single-channel lifetime. Addition of polylysine does not affect τ for gA channels, but causes a substantial increase in τ for OPg channels. This effect is reversed by addition of polyacrylic acid. The deceleration of the photoinactivation kinetics is ascribed to electrostatic interaction of polylysine with OPg probably resulting in OPg clustering. The latter can stabilize the channel state by reducing the rotational and lateral mobility of OPg monomers and dimers, and thus increase the single channel lifetime.

Key words: Gramicidin channel; Polylysine; Bilayer lipid membrane; Cluster; Domain; Photosensitizer

1. Introduction

Synthetic polyelectrolytes are widely used now in medicine and biology [1–3] that requires studying their influence on a cell and its components. In this connection, it is useful to study the interaction of the polyelectrolytes with an artificial bilayer lipid membrane (BLM) as a model of a cell membrane. The adsorption of a synthetic polycation on a negatively charged lipid membrane can be accompanied by modification of membrane structure and lipid mobility [4–7], in particular, by an increase in the gel-to-liquid-crystalline phase transition temperature [8–11], lateral phase segregation [8–12], transmembrane diffusion of lipid molecules [13,14] and an increase in membrane permeability to inorganic ions [15,16]. A series of studies dealt with interaction of charged oligopeptides with membrane lipids [7,17,18], in particular, experimental results and theoretical models proved its electrostatic nature [19, 20].

It is reasonable to assume that a polycation being adsorbed on a cell membrane should affect functioning of membrane proteins. However, there are only few publications on this matter. For example, it has been shown that polycations can affect ion permeation through the channels formed by alamethicin in BLM [21]. According to [22], polylysine acts as a trigger of the calcium channel in the ryanodine receptor of the sarcoplasmic reticulum producing a conformational change in the protein. The present study deals with the effect of the cationic polypeptide, polylysine, on the ionic channels formed by pentadecapeptide gramicidin A and its negatively charged analogue *O*-pyromellitylgramicidin in BLM. The kinetic properties of these channels studied by the method of sensitized photoinactivation [23] are shown here to change significantly upon the addition of polylysine to BLM prepared from neutral lipids.

2. Materials and methods

BLMs were formed from a solution of 2% diphytanoylphosphatidylcholine (DPhPC) (Avanti Polar Lipids) in *n*-decane (Merck) by the brush technique [24] on a 0.55-mm diameter hole in a Teflon partition separating two compartments of a cell. Both compartments were filled with aqueous solutions of 100 mM KCl (Fluka), 10 mM MES (Sigma), 10 mM Tris (Sigma), 0.05 mM EDTA (Sigma), pH 7.0. Gramicidin (Sigma) and *O*-pyromellitylgramicidin (OPg) (a gift of Prof. R.E. Koeppe, II) synthesized as described in [25] were added from stock solutions in ethanol to the bathing solutions at both sides of the BLM and routinely incubated for 15 min at constant stirring. Poly-(L-lysine) (M_r 12 000; degree of polymerization 60; Sigma) and polyacrylic acid (M_r 250 000; degree of polymerization 3500; Aldrich) were added to both compartments of the cell unless otherwise stated.

A photosensitizer, aluminium trisulfophthalocyanine ($AlPcS_3$), kindly provided by Dr. M.G. Galpern, was added to the bathing solution at the *trans* side (the *cis* side is the front side with respect to the flash lamp).

The electric current (I) was recorded under voltage-clamp conditions. The currents were measured by means of a home-made amplifier, digitized by DT2814 (Data Translation) and analyzed by a personal computer. Ag-AgCl electrodes were put directly into the cell, the voltage of 30 mV was applied to the BLM. BLMs were illuminated by single flashes produced by a xenon lamp with flash energy of about 0.3 J and flash duration < 2 ms.

Experiments were carried out at room temperature (20–22°C).

3. Results and discussion

It has been shown previously that the time course of inactivation of the gramicidin-mediated current through BLM caused by a flash in the presence of a photosensitizer (sensi-

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Abbreviations: DPhPC, diphytanoylphosphatidylcholine; gA, gramicidin A; OPg, *O*-pyromellitylgramicidin; $AlPcS_3$, aluminium trisulfophthalocyanine; BLM, bilayer lipid membrane

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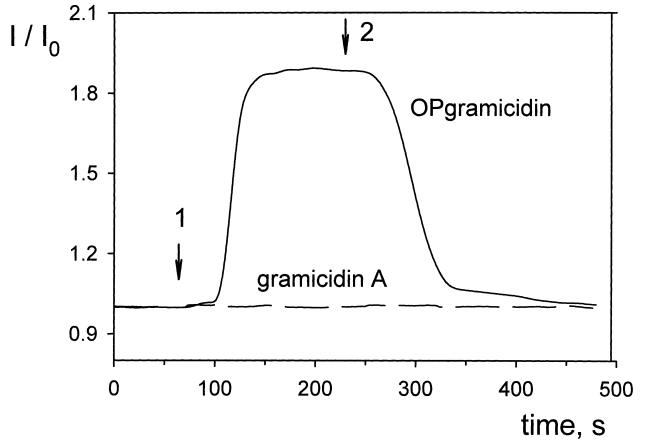

Fig. 1. Effect of the addition of polylysine (0.09 μM) at the moment marked by arrow 1 on the *O*-pyromellitylgramicidin-mediated current (solid curve) and gramicidin A-mediated current (dashed curve) through BLM. At the moment marked by arrow 2, 7 nM polyacrylic acid is added. I_0 is 2 μA.

tized photoinactivation), reflects equilibration of gramicidin monomers and dimers after the sensitized photodamage of a portion of the monomers [23]. It should be noted that a transmembrane dimer is generally assumed to represent the channel state of gramicidin in BLMs [26]. The kinetics of gramicidin photoinactivation is dependent on temperature and characteristics of a membrane: for instance, chemical nature of solvent used in the membrane-forming solution [23] and dipole potential of BLM [27]. The time course of photoinactivation in most cases is described by a single-exponential decay with an exponential factor (τ, the characteristic time of photoinactivation) which correlates well with the single-channel lifetime. Here the method of sensitized photoinactivation is employed for studying the effect of polylysine on gramicidin channels.

It has been supposed that attachment of a negatively charged group to the carboxyl terminus of gramicidin which is located near the channel mouth may give rise to interaction of gramicidin with positively charged polylysine. Indeed, addition of polylysine to the bathing solutions (marked by arrow 1 in Fig. 1) leads to an increase in the current through BLM mediated by OPg (Fig. 1, solid curve), the gramicidin analogue having three negatively charged groups at the C-terminus [25,28]. This effect is completely reversed by the addition of polyacrylic acid (arrow 2, Fig. 1). Under these conditions, the concentration of negatively charged monomeric units of polyacrylic acid is in excess to the concentration of positively charged monomeric units of polylysine. No change in the current is observed in the case of usual gramicidin, gA (Fig. 1, dashed curve). In this connection we have performed experiments on sensitized photoinactivation of OPg channels. In all the experiments, the channels were incorporated into BLMs formed of neutral lipids (DPhPC) which excludes the electrostatic interaction of polylysine with lipids.

Fig. 2A shows the kinetics of the flash-induced decrease in OPg-mediated conductance of BLM in the presence of the photosensitizer before (curve 1) and after the addition of polylysine to the bathing solution at one side of BLM (curve 3) and at both sides (curve 2). It is seen that in the latter case the addition of polylysine leads to a marked deceleration of the photoinactivation kinetics and a decrease in its amplitude, namely τ increases from 0.6 to 3.2 s, while the amplitude is reduced by 40%. The deceleration is resolved much better when the kinetics is presented in a semilogarithmic plot as $\ln(I-I_\infty)/(I_0-I_\infty)$ versus time, because the slope of the kinetic curves is proportional to $1/\tau$ in the case of the exponential decay of the current (I_∞ is the stationary value of the current and I_0 is the initial value of the current, see inset to Fig. 2A). Subsequent addition of polyacrylic acid completely reverses the effect of polylysine (curve 4). Polylysine does not produce any effect on photoinactivation of gA (Fig. 2B with the inset).

The data obtained indicate that the influence of polylysine on the conductance and the photoinactivation kinetics of OPg channels in BLM results from adsorption of polylysine on the negatively charged DPhPC/OPg membrane due to formation of ionic contacts between positive lysine units and negative C-terminal groups of OPg. Electrostatic binding of linear syn-

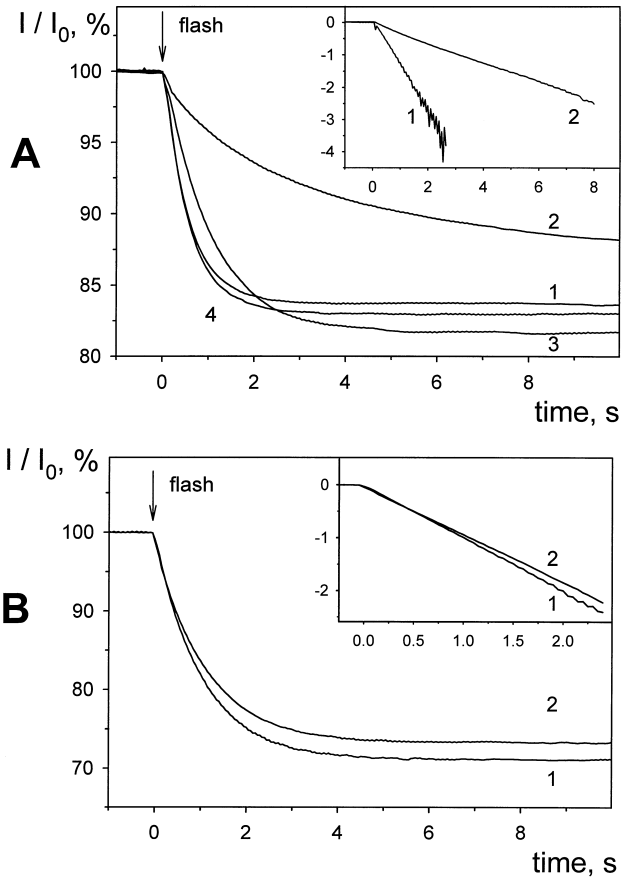

Fig. 2. A: The effect of polylysine on the kinetics of the decrease in the *O*-pyromellitylgramicidin-mediated current (*I*) through BLM after a flash of visible light (at zero time) in the presence of 1 μM $AlPcS_3$. Polylysine (0.35 μM) is added at one side of the BLM (curve 3) or at both sides (curve 2). Curve 1 is the control in the absence of polylysine. Curve 4 shows the current trace after the addition of 0.14 μM of polyacrylic acid. The normalized values of the current (I/I_0) are plotted vs. the time. The initial value of the current (I_0) is approximately 3 μA. Inset: The data presented as $\ln(I-I_\infty)/(I_0-I_\infty)$ vs. the time where I_∞ is the stationary value of the current. B: The effect of polylysine on the kinetics of the decrease in the gramicidin A-mediated current (*I*) through BLM after a flash of visible light (at zero time) in the presence of 1 μM $AlPcS_3$. Polylysine (0.18 μM) is added at both sides (curve 2) of the BLM. Curve 1 is the control in the absence of polylysine. The normalized values of the current (I/I_0) are plotted vs. the time. The initial value of the current (I_0) is approximately 3 μA.

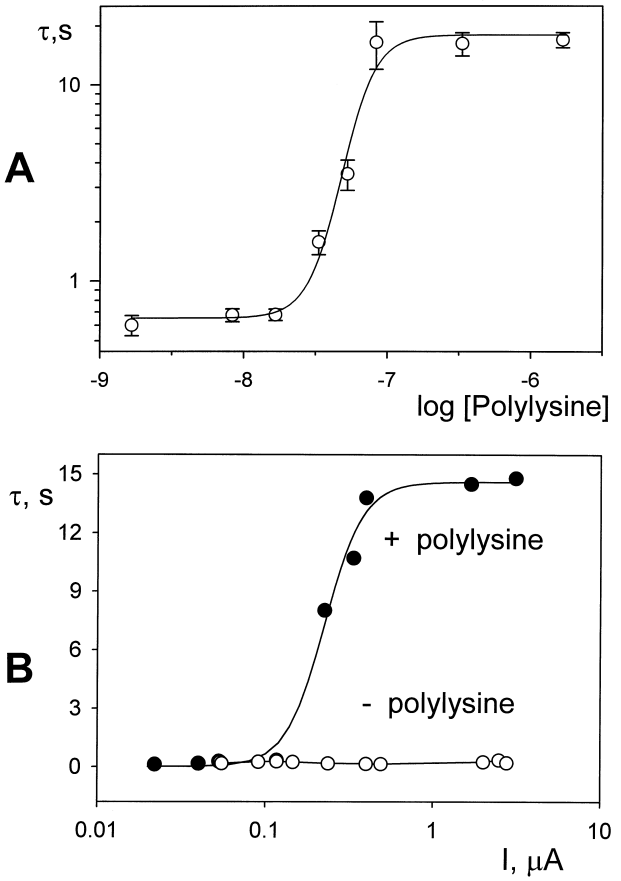

Fig. 3. A: The dependence of the characteristic time of *O*-pyromellitylgramicidin photoinactivation (τ) measured in the presence of polylysine on its concentration. The initial value of the current for each measurement of τ is approximately 3 μA. B: The dependence of the characteristic time of *O*-pyromellitylgramicidin photoinactivation (τ) on the initial current through BLM in the presence of polylysine (0.09 μM) (closed circles), and in control, in the absence of polylysine (open circles).

thetic polycations to negatively charged BLMs was detected, for example, by changes in membrane surface potential [29]. The reversal of the effect of polylysine on the OPg photoinactivation kinetics produced by addition of polyacrylic acid (Fig. 2A) is apparently explained by the formation of a negatively charged interpolyelectrolyte complex between anions of polyacrylic acid and adsorbed polylysine chains, followed by removal of the complex from the negatively charged DPhPC/OPg membrane into the solution. Complete removal of polycations from negatively charged membranes of liposomes caused by recomplexation with an excess of linear polyanions has been shown in [14–16,30–32].

The fact that polylysine causes the increase in the characteristic time of photoinactivation only in the case of the negatively charged gramicidin analogue (compare Fig. 2A and B) proves that it is the direct interaction of polylysine with the channel-forming peptide that is responsible for the effect on the kinetics of photoinactivation.

In Fig. 3A the characteristic time of OPg photoinactivation is plotted versus the concentration of polylysine in the bathing solution. It is seen that a progressive increase in τ from 0.6 s to 12 s occurs in a rather narrow range of polylysine concentration (between 1.8×10^{-8} and 9×10^{-8} M), whereas τ remains constant when the concentration exceeds 9×10^{-8} M.

The data presented in Fig. 3A support the assumption that the deceleration of the photoinactivation kinetics is associated with binding of polylysine to OPg. It is seen that at low concentrations of polylysine the effect on τ is negligible. It appears only at a threshold concentration which is presumably sufficient to induce segregation of OPg molecules into domains. The sigmoidal increase in τ between 2.3×10^{-8} M and 9.2×10^{-8} M polylysine apparently reflects cooperativity in polylysine binding to OPg caused by formation of OPg domains. It should be noted that formation of extended rafts by gA monomers has been suggested to explain the single channel data under certain conditions [33].

In contrast to the data on the effect of polylysine, it has appeared that the addition of Ca^{2+} at concentrations up to 60 mM does not alter the kinetics of OPg photoinactivation in BLMs formed of DPhPC (compare with the data on Ca^{2+}-induced reduction of the lifetime of gA channels in the mem-

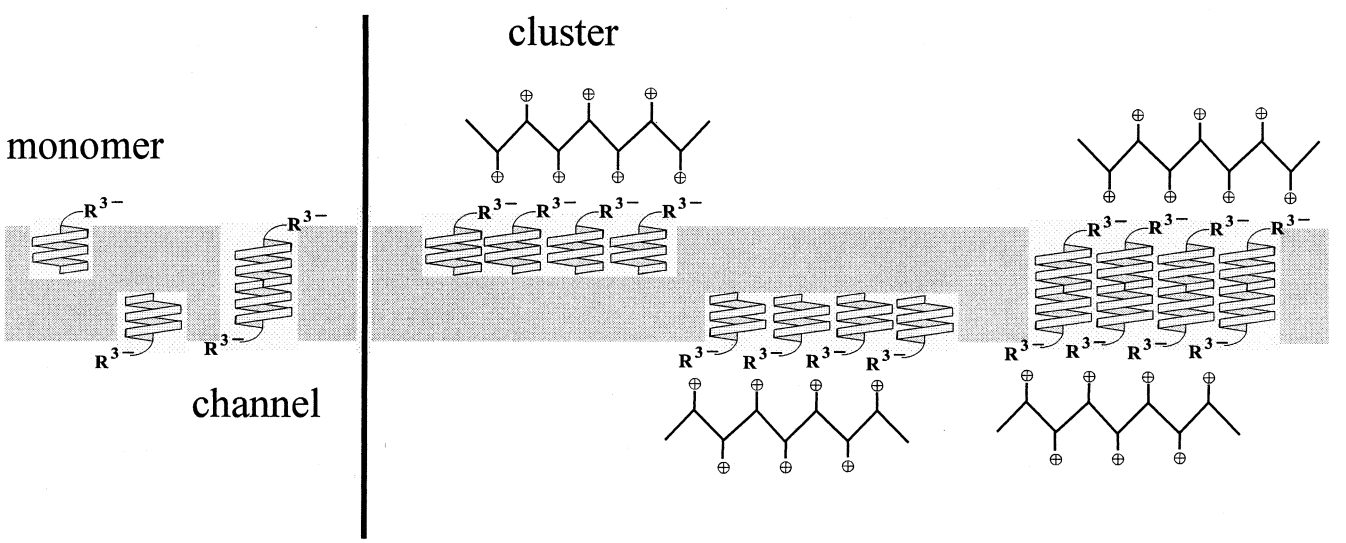

Fig. 4. Scheme of the channel formation by *O*-pyromellitylgramicidin in the absence (left side) and in the presence (right side) of polylysine.

branes formed of dioleoylphosphatidylserine [34]). It is worth noting that addition of DL-lysine at concentrations up to 10 mM does not affect the characteristic time of OPg photoinactivation (data not shown).

As seen from Fig. 3B, the effect of polylysine on τ is dependent on the value of the OPg-mediated current, i.e. on the concentration of OPg, since the current through BLM is known to be proportional to the square of the OPg concentration [28]. Fig. 3B shows that τ measured in the presence of 9×10^{-8} M polylysine is practically constant at high values of the current and steeply decreases when the current is reduced below the critical value, so that at sufficiently low current the addition of polylysine does not bring about any deceleration of the photoinactivation kinetics.

The above data can be explained in terms of the hypothesis of the polylysine-induced clustering of OPg molecules in BLM. It can be supposed that each macromolecule of adsorbed polylysine binds electrostatically to several OPg molecules thereby forming a cluster (see scheme in Fig. 4). Clustering of OPg leads to stabilization of the channel state (transmembrane dimer) by reducing the lateral and rotational mobility of OPg in the membrane. At high concentrations of polylysine, all the OPg molecules in the membrane are presumably involved in the formation of clusters which explains the saturation of the concentration dependence observed above 9.2×10^{-8} M polylysine (Fig. 3A). The fact that the increase in τ induced by polylysine is diminished abruptly upon lowering the OPg-mediated conductance of BLM (Fig. 3B) confirms the idea that the deceleration of the photoinactivation kinetics is due to OPg clustering. Obviously the binding of polylysine which induces the formation of OPg clusters becomes impossible at low concentrations of OPg.

The great enhancement of the effect of polylysine on the kinetic properties of OPg channels upon the symmetrical as compared to asymmetrical addition of polylysine to the bathing solutions (Fig. 2A) shows that dramatic retardation of the OPg dimer-monomer equilibration kinetics occurs if polylysine molecules adsorb on both leaflets of BLM which apparently induces clustering of OPg molecules at both sides of the membrane.

As to the effect of polylysine on the amplitude of photoinactivation, it cannot be attributed simply to quenching of reactive oxygen species, because lysine is known to be a poor quencher (see [35] and references therein). It should be mentioned that the pronounced decrease in the amplitude of OPg photoinactivation takes place only when polylysine is added at both sides of BLM (Fig. 2A) which is not expected with quenching of reactive oxygen species. More likely, the reduction of the amplitude of photoinactivation is due to a decrease in accessibility and/or sensitivity of OPg molecules to reactive oxygen species after polylysine binding.

In conclusion, it should be noted that the results of the present work have demonstrated the significant stabilization of gramicidin channels induced by their interaction with polycations which can be attributed to channel clustering.

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