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Actin and amphiphilic polymers influence on channel formation by Syringomycin E in lipid bilayers

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Abstract The bacterial lipodepsipeptide syringomycin E (SRE) added to one (*cis*-) side of bilayer lipid membrane forms voltage dependent ion channels. It was found that G-actin increased the SRE-induced membrane conductance due to formation of additional SRE-channels only in the case when actin and SRE were applied to opposite sides of a lipid bilayer. The time course of conductance relaxation depended on the sequence of SRE and actin addition, suggesting that actin binds to the lipid bilayer and binding is a limiting step for SRE-channel formation. G-actin adsorption on the membrane was irreversible. The amphiphilic polymers, Konig's polyanion (KP) and poly(Lys, Trp) (PLT) produced the actin-like effect. It was shown that the increase in the SRE membrane activity was due to hydrophobic interactions between the adsorbing molecules and membrane. Nevertheless, hydrophobic interactions were not sufficient for the increase of SRE channel-forming activity. The dependence of the number of SRE-channels on the concentration of adsorbing species gave an *S*-shaped curve indicating cooperative adsorption of the species. Kinetic analysis of SRE-channel number growth led to the conclusion that the actin, KP, and PLT molecules form aggregates (domains) on the *trans*-monolayer. It is suggested that an excess of SRE-channel formation occurs within the regions of the *cis*-monolayer adjacent to the domains of the adsorbed molecules, which increase the effective concentration of SRE-channel precursors.

Keywords Actin · Syringomycin E · Channel formation · Protein–lipid interactions

Abbreviations DPhPC: 1,2-Diphytanoyl-*sn*-glycero-3-phosphocholine · DOPS: 1,2-Dioleoyl-*sn*-glycero-3-phosphoserine · DOPE: 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine · DOPS/DOPE: Equimolar mixture of DOPS and DOPE · SRE: Syringomycin E · KP: Konig's polyanion · PLT: Copolymer of lysine and tryptophan

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Introduction

Interactions between the cytoskeleton and the plasma membrane are rigorously studied. Actin is an abundant component of the cytoskeleton, and it forms a large-scale submembrane filamentous network. Reorganizations of the actin cytoskeleton and its interactions with the plasma membrane in response to various stimuli appear to be important in many cellular processes, including motility, endocytosis, secretion and ion transport. It is known that the actin cytoskeleton regulates epithelial sodium channels (Berdiev et al. 1996, 2001), channels of cystic fibrosis receptor (Ismailov et al. 1997) and VDAC channels (Xu et al. 2001). Despite intensive studies, the detailed mechanisms of intracellular adhesion between the actin cytoskeleton and the plasma membrane are still not well understood.

Two types of interactions between actin and membrane bilayers are documented. The first is actin binding to the membrane with the help of docking proteins such as spectrin and vinculin (Isenberg and Niggli 1998). The second is the direct interaction between actin and lipid bilayers via electrostatic and hydrophobic forces (St-Onge and Gicquaud 1989, 1990). Although shown in vitro, this type of actin-membrane attachment can occur in vivo

(Gicquaud 1995). Pronounced interactions between F-actin and lipid bilayers reported by Grigoriev et al. (2000) include actin adsorption on bilayer surfaces and its transmembrane penetration to produce pore-like defects.

The small cationic lipodepsipeptide syringomycin E (SRE) plays important roles in the interactions of *Pseudomonas syringae* with plants (Bender at al. 1999). The plasma membranes of fungi and plants are the major targets for this toxin. At one (*cis*-) side addition to lipid bilayer, SRE forms well-defined ion channels of large and small conductance and the "large" channels are clusters of the "small" ones (Kaulin et al. 1998). Both kinds of channels are anion selective and possess pronounced voltage gating (Feigin et al. 1996; Schagina et al. 1998). SRE produces an asymmetric pore with the *trans*-opening formed from host bilayer lipids (Malev et al. 2002). It was found that SRE-channel formation is affected by actin only in the case when actin and SRE are applied to opposite sides of a lipid bilayer (Gurnev et al. 2003; Bessonov et al. 2004). In other words, actin influences SRE-channel formation by interacting with that side of the membrane (*trans*-side), where the "lipidic" part of the SRE-channel is formed. The SRE-channel conductance, voltage gating, and channel-forming activity of SRE (i.e. the ability of the toxin to form channels in a bilayer) are influenced by bilayer lipid composition, cholesterol content, lipid charge, lipid aggregation state, and dipole-modifying species (Feigin et al. 1997; Gurnev et al. 2002; Malev et al. 2002; Schagina et al. 2003). These features make SRE an attractive probe to monitor the state of lipid bilayer upon modification of some potentially membrane-active species. Likewise, the channel-forming properties of gramicidin A were used to detect the Ca^{2+}-dependent association of annexins with lipid bilayers (Eskesen et al. 2001), and to clarify the role of amphipathic compounds on the mechanical properties of membranes (Suchyna et al. 2004).

In the current work, the influence of G-actin and amphiphilic polymers: Konig's polyanion (KP) and the positively charged copolymer of lysine and tryptophan (PLT) on the channel-forming activity of SRE in planar lipid bilayers was examined. It was established that the dependencies of the number of SRE-channels on the *trans*-concentration of actin, KP, and PLT are of a sigmoid form, suggesting a cooperative adsorption of these molecules on the membrane surface. We speculate that adsorbing molecules form some aggregates (domains) in the *trans*-monolayer of membrane and the excess of SRE-channel formation occurs within the regions of the *cis*-monolayer adjacent to the aggregates in the *trans*-monolayer of the membrane.

Materials and methods

Lipids used in this study, synthetic 1,2-dioleoyl-*sn*-glycero-3-phosphoserine (DOPS), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) and 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DPhPC) were purchased from Avanti Polar Lipids, Inc. (Pelham, AL, USA). All electrolytes were of reagent grade (Sigma, St. Louis, MO, USA). Water was deionized and double distilled. Salt solutions for bilayer experiments were in the range of 0.1–1.0 M NaCl. All solutions were buffered by 5 mM MOPS to pH 6.0. Syringomycin E was purified as described previously (Bidway et al. 1987). Copolymer of L-lysine and L-tryptophan (at ratio 4:1) (molecular weight ≈38 kDa) and trypsin (from bovine pancrease) were purchased from Sigma (St. Louis, MO, USA). Konig's polyanion (copolymer of methacrylate, maleate and styrene in 1:2:3 ratio with molecular weight ≈10 kDa) was obtained courtesy of N.S. Melik-Nubarov (Moscow State University). Polyanion structure and synthesis procedures were previously described (Konig et al. 1982).

Rabbit skeletal muscle actin was purified by the standard procedure (Pardee and Spudich 1982). Actin was purified by two cycles of polymerization–depolymerization, using 30 mM KCl for polymerization. The *A* parameter was used to probe the quality of actin (Turoverov et al. 1976). *A* was defined as $A = (I_{320}/I_{365})_{297}$, where I_{320} and I_{365} are fluorescence intensities at wavelength λ_{em} = 320 and 365 nm. Only samples of G-actin with $A > 2.56$ (content of inactivated actin not higher than 2–3%, (Turoverov et al. 1976)) were used. The actin concentration was determined with a Hitachi spectrophotometer (Japan) using a molar extinction of $E_{280}^{1\,cm} = 1.09$ (Rees and Young 1967). G-actin in buffer (0.2 mM ATP, 0.1 mM $CaCl_2$, 0.4 mM α-mercaptoethanol, 5 mM Tris–HCl, pH 8.2, 1 mM NaN_3) was stored on ice and used within a week. It should be added that G-actin concentrations used in the study were below the critical concentration for actin polymerisation in an aqueous medium (Sheterline et al. 1999). For proteolytic cleavage, actin and trypsin solutions were mixed at ratio 4:1 (on weight basis) and incubated for 1 h at room temperature. Proteolysis leads to formation of two fragments (about 33 and 9 kDa (Mornet and Ue 1984)). The degree of actin proteolysis was visualized using SDS-polyacrylamide gel electrophoresis.

Virtually solvent-free membranes were formed as described by Montal and Mueller (1972). Un- and negatively-charged membranes were formed from DPhPC or equimolar mixture of DOPS and DOPE (DOPS/DOPE), respectively. Two symmetrical compartments of a Teflon chamber each with a solution volume of 1.5 cm^3 were separated by a 15 μm-thick Teflon partition containing a round aperture of ~100-μm diameter. Hexadecane was used for aperture pretreatment. Ag/AgCl electrodes connected with the membrane bathing solution via 200 μl pipette tips filled with 1.5% agarose in 2 M KCl were used to detect ion currents. The term "positive voltage" means that the *cis*-side compartment (the side of SRE addition) is positive with respect to the *trans*-side. SRE was added to the aqueous phase from water stock solutions (1 mg/ml, pH 3) after bilayer

formation. The mean values of the current through single small channels were obtained from current histograms. For each current level, amplitude histogram was fitted with the Gaussian distribution using Origin software (Microcal Software, Inc., Northampton, MA, USA). The effective gating charge of the SRE-channel (Q) was measured in voltage-jump experiments (Malev et al. 2002). To determine the number of open SRE-channels at a given membrane potential, the steady-state current was divided by the corresponding voltage-dependent current of a single small channel. Actin, KP and PLT by themselves had no pore-forming activity at the concentrations that were used in the experiments with SRE.

Unless otherwise noted, bilayers from equimolar mixture of DOPS and DOPE and bathed in 0.1 M NaCl, pH 6 were used. In kinetic experiments (measurements of current relaxation after a transmembrane voltage step) voltage, V, was equal to ± 50 mV. All experiments were performed at room temperature.

Results and discussion

Effects of *cis*- and *trans*-addition of actin, KP and PLT on the SRE-induced conductivity

The influence of G-actin on the channel-forming activity of SRE is illustrated in Fig. 1. Upon membrane formation, SRE was first added to the electrolyte solution of the *cis*-compartment of the chamber at a concentration corresponding to formation of several single channels at the applied voltage of 50 mV. Subsequent *cis*-side actin addition did not affect the SRE activity, but *trans*-side addition produced a pronounced increase in the membrane current. Reversing the voltage polarity produced channel closure, typical for SRE-channels (Malev et al. 2001). The single-channel current for small channels did not depend on actin addition to the *trans*-side of the bilayer (Fig. 1, insets). The estimated value of effective gating charge, Q, of the SRE-channel in the presence of G-actin (DOPS/DOPE bathed in 0.1 M NaCl, pH 6) was equal to unity ($Q = 1.04 \pm 0.14$); the same value was previously reported for actin-free systems ($Q = 1.08 \pm 0.07$) (Malev et al. 2002). Since SRE-channel conductance and Q remain the same in both the presence and the absence of actin, these findings indicate that actin *trans*-side addition stimulates the SRE channel-forming activity rather than forming other conducting units. This is supported by the following facts. The time needed for steady-state current relaxation was 130 ± 40 min for G-actin (see for example Fig. 1) and 200 ± 20 min for F-actin (Bessonov et al. 2004) at actin concentrations in the solution of the *trans*-compartment equal to 4 μg/ml. Without actin, 9 ± 3 min were necessary to achieve the steady-state number of open SRE-channels (Fig. 2a). So, adsorption of actin is likely to be a limiting step in the observed SRE steady-state current relaxation. Assuming that actin binding to lipid bilayers is a time-limiting step, it was speculated that addition of SRE into the system where the equilibrium of actin binding was already achieved would produce a faster increase of the SRE-induced current. Indeed, when G-actin was applied 150 min prior to SRE application, only 9 ± 3 min were enough to reach the steady-state current (Fig. 2b), i.e. the same time as in the absence of the actin. These findings show that the observed increase of the transmembrane current by *trans*-addition of actin (Fig. 1) results in the increase in the number of SRE-channels. This process is limited by either a slow adsorption of actin, or some slow process initiated by its adsorption. It, therefore, seemed that the transition from

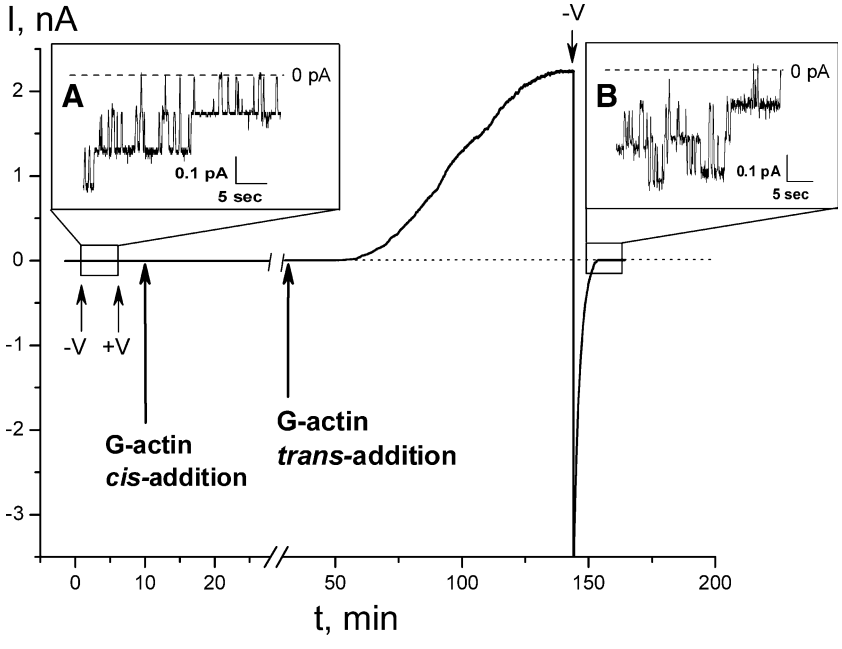

Fig. 1 Typical time courses of the SRE-induced transmembrane current with addition of actin. SRE was first added to the *cis*-side of the lipid bilayer (0.7 μg/ml) to provide the stable formation of several channels. Subsequent application of G-actin (4 μg/ml) to the *cis*-side did not produce measurable changes in membrane conductance, while *trans*-side actin addition initiated current increase. Reversing the voltage sign stimulated current decrease. Single-channel amplitudes, resolved at the end of the recording (**b**), did not diverge from those observed before actin addition (**a**). DOPS/DOPE membranes were bathed in 0.1 M NaCl, pH 6 (5 mM MOPS/NaOH). Applied voltage was $\pm$ 50 mV

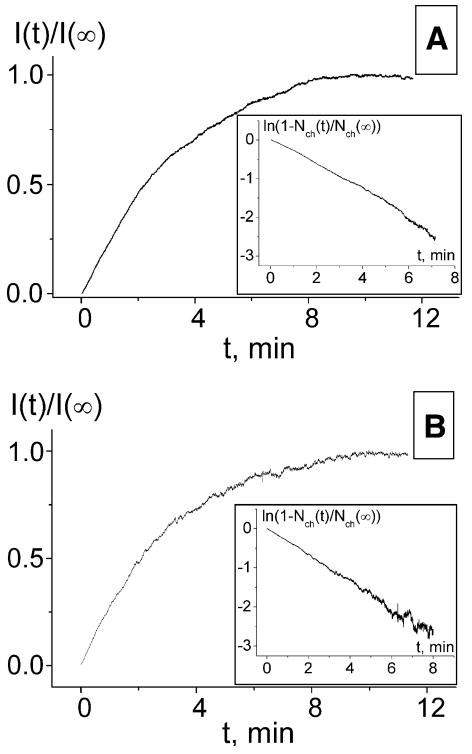

Fig. 2 Kinetics of normalized SRE-induced transmembrane currents ($I(t)/I(\infty)$) without (**a**) and with addition of G-actin (**b**). G-actin was added to the *trans*-side 150 min before SRE addition. Data were obtained for DOPS/DOPE bilayers bathed by 0.1 M NaCl at pH 6. The concentration of G-actin was 4 μg/ml. SRE concentrations were 6 μg/ml (**a**), and 1 μg/ml (**b**). Insets illustrate linear dependence of $\ln[1-N_{ch}(t)/N_{ch}(\infty)]$ on time

an actin-free to actin-equilibrated membrane would be accompanied with changes in the kinetics of opening/closure of SRE-channels at application of the same V.

In earlier studies, it was established that the opening/closure of SRE-channels results from the transition of a channel precursor from a non-conductive state to a conductive one (Malev et al. 2001). A simple first order differential equation describes the kinetics of the opening/closure of SRE-channels, at least in the first approximation, as follows:

$$dN_{ch}(t)/dt = kN_{pr} - \rho N_{ch}(t) \quad (1)$$

Here, k and ρ are the rate constants of SRE-channel opening and closure, respectively; $N_{ch}(t)$ is the number of open channels at moment t; and N_{pr} is the concentration of channel precursors at the membrane surface. The solution of Eq. 1 satisfying the initial condition: $N_{ch}(0) = 0$ is as follows:

$$\ln[1 - N_{ch}(t)/N_{ch}(\infty)] = -\rho t \quad (2)$$

Here, $N_{ch}(\infty)$ is the steady-state number of open channels. This equation is in accord with the results obtained for membranes modified with SRE (Fig. 2a, inset) and also equilibrated with actin (Fig. 2b, inset). The rate constant ρ obtained from the slope of the curves did not significantly change at the transition from actin-free to actin-equilibrated systems ($\rho = 0.30 \pm 0.03$ and 0.26 ± 0.04 min^{-1}, correspondingly). Thus, no changes in the mechanism of opening/closure of SRE-channels occurred at the indicated transition. At the same time, changes in the number of SRE-channels after the *trans*-side actin addition exceeded up to three orders of magnitude under the same concentration of SRE in *cis*-compartment of the bathing electrolyte. Beside the rate constant ρ that is independent of the actin concentration, Eq. 1 includes the precursor concentration N_{pr} and the rate constant k, which could be changed by actin addition. Hence, two explanations for the observed effect of actin are possible. Firstly, actin's adsorption on the *trans*-side of the bilayer increases the precursor concentration, by changing the affinity of the membrane *cis*-monolayer to the SRE-channel precursors. Secondly, the relevant increase of the rate constant k might also be induced by adsorbed actin particles.

Based on the above considerations, one can introduce an "effective" concentration of precursors, N^*_{pr}, defined by the following relation:

$$N^*_{pr} = [k(C_a)/k]N_{pr}(C_a) \quad (2a)$$

where k is the rate constant in the absence of actin, while a possible dependence of the rate constant and the precursor concentration on the concentration of adsorbed particles (C_a) in *trans*-compartment is taken into account by using symbols $k(C_a)$ and $N_{pr}(C_a)$, respectively. Substitution of N^*_{pr} instead of N_{pr} into Eq. 1 does not change this equation, but indicates the influence of adsorbed actin particles on N^*_{pr} without implying a mechanism. To indicate the possible complex influence of actin addition on the effective concentration of N^*_{pr}, the term "affinity" is further used so that adsorption of actin on the *trans*-side of the bilayer increases concentration N^*_{pr} by changing the "affinity" of *cis*-monolayer to the precursors of SRE-channels.

The adsorption of actin to lipid bilayers might result from electrostatic and/or hydrophobic interactions (Bouchard et al. 1998; St-Onge and Gicquaud 1990). To elucidate the nature of protein–lipid interactions responsible for the increase of SRE channel-forming activity, the effect of different synthetic polymers on the activity of SRE was tested. *Trans*-side addition of negatively charged poly-L-glutamic acid to the SRE-modified negatively charged bilayers bathed in 0.1 M NaCl (pH 6) did not affect the activity of SRE. The addition of the polycation, did not also increase the SRE channel-forming activity. Moreover, poly-L-lysine addition to the *trans*-side of the bilayer produced a considerable loss of SRE-channel formation (Gurnev et al. 2003). These data decline the role of electrostatic interactions in the rise of SRE channel-forming activity.

On the other hand, the number of SRE-channels in DOPS/DOPE membranes bathed in 0.1 M NaCl (pH 6) increases in the case of *trans*-addition of amphiphilic polymers: KP or PLT (data not shown). It was shown

that KP binding to lipid bilayers resulted from hydrophobic interactions between the polymer and bilayer (Maltseva et al. 2002). For these substances, no change was found in the value of rate constant ρ of the kinetic dependence given by Eq. 2 for equilibrium conditions of their adsorption and in their absence, as it was illustrated for G-actin (see Fig. 2). We also observed an increase in the number of SRE-channels in the presence of actin for the systems where electrostatic protein–lipid interaction was significantly reduced, i.e. for the negatively charged membranes bathed by 1 M NaCl (pH 6) and for uncharged (DPhPC) membranes (0.1 M NaCl, pH 6) (data not shown). The similar data were the reasons why the effect of F-actin on the SRE-channel forming activity was attributed to the hydrophobic protein-lipid interactions (Bessonov et al., 2004). Thus, these results emphasize the importance of the hydrophobic interactions between actin, KP and PLT and lipid bilayer in the increase of the SRE channel-forming activity. The existence of the hydrophobic interactions suggests some degree of penetration of substances under investigation into the inner part of *trans*-monolayer. This action might change the local structure of the *cis*-monolayer within the region adjacent to the adsorbed particles and, thus, increase the "affinity" of SRE-channel precursors to that monolayer.

Nevertheless, other cytoskeletal proteins, as vinculin and alpha-actinin, which interact with membrane through hydrophobic interactions (Niggli et al. 1986; Han et al. 1997; Goldmann et al. 1999; Niggli 2001) did not affect the SRE channel-forming activity. The same results were obtained in the case of *trans*-addition of myoglobin, albumin, and lysozyme, the substances that are able to adsorb on lipid membranes due to hydrophobic interactions (Buijs et al. 2003; Tsunoda et al. 2001). These data show that the presence of hydrophobic interactions between adsorbing molecules and membrane is necessary, but not sufficient to trigger the increase of the SRE channel-forming activity.

Thus, it has been established that actin in both G- and F-forms, KP, and PLT increase the SRE-channel forming activity in lipid bilayer if they are added to the opposite site of the SRE application. Although their effects are quantitatively different, a qualitative picture of their influence is the same.

Domain formation hypothesis

The above results do not explain mechanisms by which the adsorption of actin, PLT, and KP on the *trans*-side of the membrane increases the channel-forming activity of SRE. To answer this question, determinations were made of $N_{ch}(\infty)$ as a function of the concentration of G-actin, C_a, introduced into *trans*-compartment of the chamber. The curve of the $N_{ch}(\infty)/N_{ch}^{max}(\infty)$-dependence versus C_a has a sigmoid form, suggesting a cooperative character of the adsorption process (see Fig. 3). Here, $N_{ch}^{max}(\infty)$ is the maximum value of $N_{ch}(\infty)$ achieved in the limit of $C_a \rightarrow \infty$. The Frumkin adsorption isotherm (Bard and Faulkner 2001) is often used to explain qualitatively the cooperative adsorption of different substances as follows:

$$\ln[\theta/(1-\theta)] = \ln[K_d C_a] - 2a\theta \quad (3)$$

In Eq. 3, θ is the occupancy of *trans*-monolayer with the adsorbing species made equal to $N_{ch}(\infty)/N_{ch}^{max}(\infty)$; K_d is a distribution coefficient of adsorbing species (between membrane and aqueous solution); a is an attraction constant (in RT units) characterizing short-range interactions of particles in an adsorption layer. If a is negative, the interactions between neighbouring adsorbed molecules are attractive; and, vice versa, at positive a, the interactions are repulsive. The above equation was used to construct a plot of the quantity $\{\ln[\theta/(1-\theta)] - \ln C_a\}$ versus θ in order to fit the data represented in Fig. 3, and high negative values of $a \approx -2$ were obtained. Dependencies of the number of SRE-channels on the concentration of KP and PLT also gave a sigmoid curve with $a \approx -1.5$ and -2 for KP and PLT, respectively. At these values of a, one can reasonably suggest formation of some aggregates (domains) of the adsorbing species in the *trans*-monolayer of the membrane. Under these conditions, it is likely that the appearance of such domains in *trans*-monolayer induces changes within the adjacent regions (further on " *cis*-domains") of *cis*-monolayer, which in turn increases the "affinity" of precursors of SRE-channels to these modified regions.

When G-actin is partially hydrolyzed by trypsin, it loses its ability to polymerize (Jacobson and Rosenbush, 1976). Assuming that formation of the domains is analogous to the polymerization of actin molecules, we tested the effect of trypsin-hydrolyzed G-actin on the

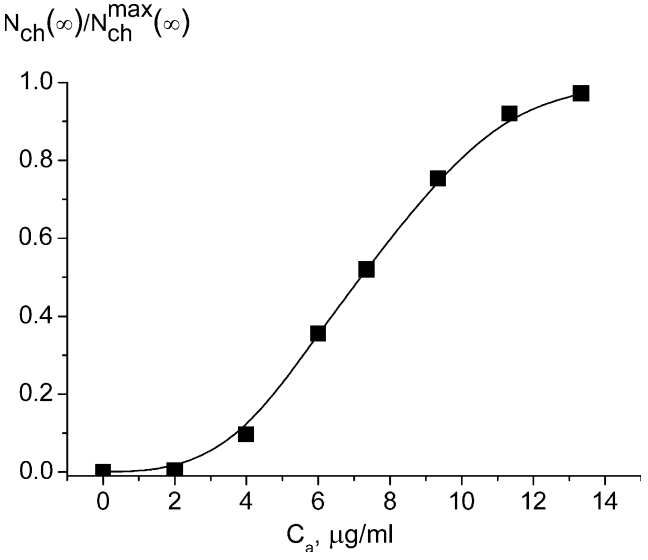

Fig. 3 Dependence of $N_{ch}(\infty)/N_{ch}^{max}(\infty)$ on G-actin concentration in the *trans*-compartment. DOPS/DOPE bilayers were bathed in 0.1 M NaCl at pH 6. The applied voltage was 50 mV. SRE (0.7 μg/ml) was added in the *cis*-compartment before G-actin addition.

channel-forming activity of SRE, and compared it with the effect of non-hydrolyzed G-actin (Fig. 4). In the case of hydrolyzed actin only a tenfold increase in the SRE-induced current was observed, while native actin led to a current increase of more than three orders in magnitude. These results support the hypothesis about the relevance of domain formation by adsorbing molecules in their effect on the number of SRE-channels. It should be added that a pronounced irreversibility of G-actin adsorption was observed; when actin molecules were previously adsorbed on the membrane, trypsin addition to the G-actin containing compartment do not reduce the actin effect to the level of its hydrolyzed state (Fig. 4). Conformational changes of actin upon binding to the bilayer, in particular upon domain formation, might prevent trypsin's accessibility to hydrolyzable regions of actin.

In Fig. 1, one can see the existence of a latent period that can be characterized by the time necessary for beginning of essential increase of the $N_{ch}(t)$-number. This period was determined as shown graphically in Fig. 5. Its duration, τ, was defined by the intercept of the lines of the initial membrane conductance (dotted line, Fig. 5) and of the practically linear rise of the conductance after primary actin application (dashed line, Fig. 5). Thus, the latent period and the time of attainment of the steady-state number of SRE-channels are commensurable quantities. Measurements of the latent period in the case of G-actin give scattering values. This is due to a slow appearance of actin domains responsible for the latent period. Indeed, generation of such domains of a new phase under subcritical concentrations ($C_a \approx 2$ to 4 μg/ml) is a stochastic process resulting from

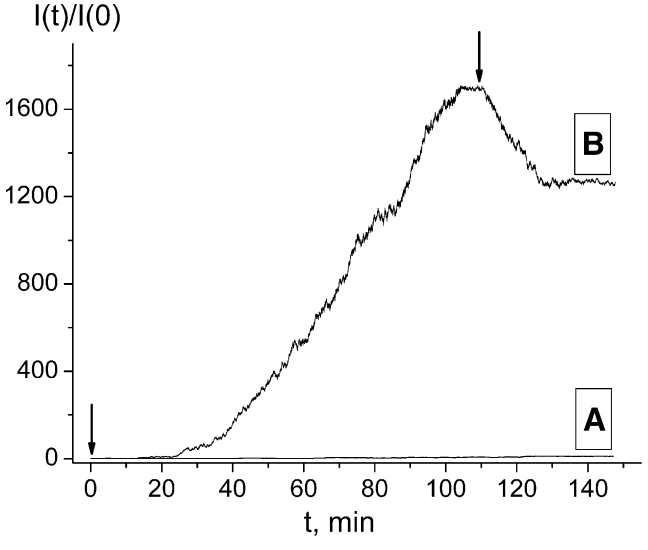

Fig. 4 Time course of $I(t)/I(0)$ with addition of trypsin-hydrolyzed (**a**) or native (**b**) G-actin. *Arrows* mark the addition of native (**b**) or hydrolyzed (**a**) G-actin at $t = 0$ and *trans*-addition of trypsin after steady-state actin-induced current achievement (**b**). Data were obtained for DOPS/DOPE bilayers bathed by 0.1 M NaCl at pH 6. The concentration of G-actin was 4 μg/ml and trypsin was 1 μg/ml. SRE concentration was 1 μg/ml

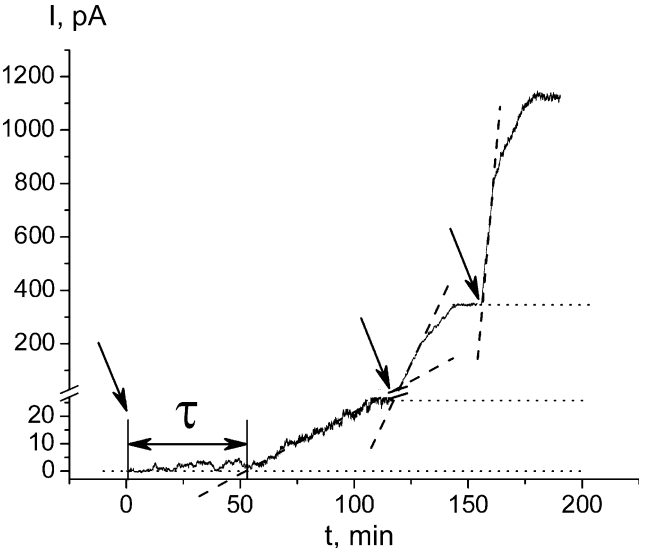

Fig. 5 Time course of the transmembrane current with three successive additions of G-actin to the *trans*-compartment. The concentrations of G-actin were 2, 4 and 6 μg/ml for the first, second, and third additions, respectively. The time period, τ, was determined as the time between actin addition (*marked by arrows*) and the beginning of the practically linear rise of the actin-induced current (indicated by *intercepts of dashed* and *dotted lines*). Values of τ were: 52, 1.3 and 1.7 min for the first, second and third additions, respectively. The SRE concentration was 1 μg/ml. Other experimental conditions were the same as those for Fig.3

accidental events in the time course. It is also evident that the probability of their generation should also depend on the concentration of actin species adsorbed at moment t of the process. Time is necessary to achieve subcritical concentrations of actin particles on the membrane to further form a domain. Thus, the observed latent time should be caused by two factors: the first is of a probabilistic nature, while the second has a deterministic one. To elucidate the role of both factors, we performed statistical determinations of the latent period at subcritical concentration $C_a = 4$ μ g/ml. The obtained histogram is depicted in Fig. 6. To fit the histogram, we used the Gaussian distribution, which gave $\tau = 30 \pm 13$ min. At the same time, one can apply the Poisson distribution to fit the obtained data, which corresponds to small numbers of newly formed domains at the membrane as a result of actin application. These results directly show a stochastic nature of the quantity under consideration, i.e. the essential contribution of the probabilistic factor to actin-induced domain formation.

Actin-induced domain formation is also supported by the following experiment. Having reached the steady-state number of open channels at a chosen concentration of G-actin, more actin was added into the *trans*-compartment, and the subsequent growth of the $N_{ch}(t)$-number was retraced. Figure 5 presents the time dependence of the transmembrane current after three consecutive *trans*-side additions of G-actin. One can see that, the *I–t* curve has an essential delay (i.e. the latent period) in the current growth after the first actin

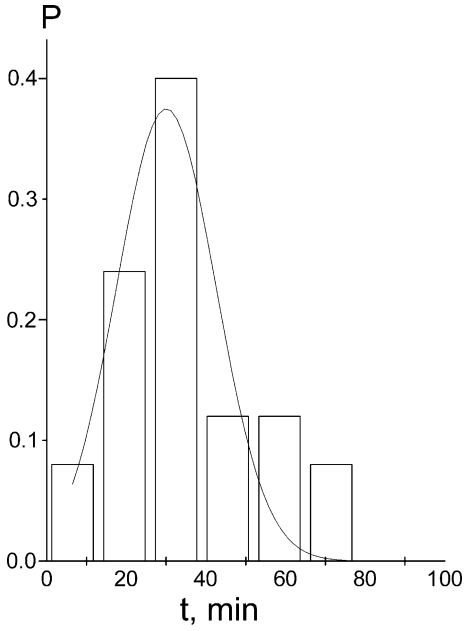

Fig. 6 Histogram of latent periods of SRE-dependent transmembrane currents measured after-it trans-side addition of G-actin. P is calculated as N/N', where N is the number of events corresponding to a particular interval (τ) ($N' = 29$). Data were obtained for DOPS/DOPE membranes bathed in 0.1 M NaCl (pH 6). The applied voltage was 50 mV. The concentration of G-actin was 4μg/ml

addition. The figure also shows the absence of the latent period with the second and following actin additions. Thus, the dependence of $I(t)$ registered at subsequent additions of actin loses its stochastic nature characteristic of the initial application of actin. This suggests that domain formation is initiated at the equilibrium concentration of adsorbed actin achieved with initial application of actin, and the observed subsequent evolution in the $N_{ch}(t)$-number reflects only an increase in the sizes of the domains. This conclusion is in qualitative agreement with the phase theory of micelle formation (Shinoda et al. 1963). According to this theory, an increase in the total concentration of aggregating particles after attainment of the critical micelle concentration results from increasing the number or sizes of micelles (here, domains) without changes in the concentration of non-aggregated particles.

The absence of latent periods after the second and subsequent additions of actin suggests either a non-limiting character of adsorption of actin particles or some change in the adsorption mechanism in the presence of actin domains. It is reasonable to suppose that the further adsorption of actin proceeds due to a direct insertion of actin particles from aqueous solution into the developing domains. In any case, the resultant time course of adsorption will be determined by the rate of increase of size of the domains and, apparently, newly formed domains. In the case of KP- and PLT-addition, the latent period is practically absent (data not shown). This probably results from the different nature of these polyions as compared to actin. In other words, unlike actin, these substances easily form domains on the *trans*-monolayer, and such domains also change the "affinity" of the SRE-channel precursors to *cis*-side of the membrane. The same concept of domain formation however seems valid for KP and PLT action, as explained below.

Further considerations

Based on the above, one can calculate the total perimeter, $2\pi\ R(t)$, of all domains appeared in the membrane up to the given moment t by using the following definition:

$$2\pi R(t) = 2\pi AM(C_a, \infty) \int_0^t r(t-\tau)P(\tau)\mathrm{d}\tau \qquad (4)$$

where $r(t-\tau)$ is the radius of a separate domain at moment $\tau \leq t$; $M(C_a,\infty)$ is the total number of domains possible under the equilibrium conditions at the unit membrane surface; A is the membrane area, and $P(\tau)$, is a distribution function that gives the probability of finding a new domain on the membrane at moment $\tau \leq t$, i.e. the distribution of domain's "birthdays" within the interval of (0, t). An analogous definition can be introduced if one is interested in the total area occupied by domains on the membrane, $\pi\ R^2(t)$, as follows:

$$\pi R^2(t) = \pi AM(C_a, \infty) \int_0^t r^2(t-\tau)P(\tau)d\tau \qquad (5)$$

Obviously, $P(\tau)$ is equal to the Dirac δ-function if the equilibrium number of domains, $AM(C_a,\infty)$, appears at the membrane at moment $t = 0$. This, in particular, corresponds to a gradual increase of actin in the *trans*-compartment after a steady-state number of open channels are achieved with initial actin addition (see above). However, a statistical representation of this quantity should be used with the initial addition of actin.

According to the above representations, one can also think that the appearance of domains on *trans*-side of the membrane instantaneously induces formation of *cis*-domains, the radius of which is equal or, at least, proportional to $r(t)$. Thus, the introduced definitions reduce the problem to calculations of the growth rate of a separate domain provided that $P(\tau)$ is known.

If the precursors are located around *cis*-domain perimeters, calculations of the total perimeter, $2\pi\ R(t)$, should be performed according to Eq. 4. If located within the *cis*-domains, the area, $\pi\ R^2(t)$, is to calculate, i.e. Eq. 5 applies. The latter is preferred, since addition of the substances (actin, KP or PLT) into *cis*-compartment of the system did not affect the number of SRE-channels suggesting some shielding from precursor formation due to the relatively large size of adsorbed particles or their repulsive interactions with SRE species. With precursor formation at the domain perimeter, such screening would not be essential.

It is further speculated that precursor formation at *cis*-domain areas determines the observed effects of actin, KP and PLT. The influences of these substances on SRE activity are high, especially in case of actin. Such large influences suggest that the precursor effective concentration within *cis*-domain areas, N^*_{pr}, is much higher than concentration N_{pr} at unmodified (by the substances in question) regions of the membrane, so that $N_{pr} \ll N^*_{pr} \neq f(t)$, which is in accord with the fact that $N^*_{pr} \neq f(t)$, for membranes treated initially with actin, KP and PLT.

Obviously, different mechanisms of domain radius growth are possible. The below quantitative analysis of these mechanisms is restricted to the cases of KP or PLT and consecutive additions of actin, where distribution function $P(\tau)$ (see Eq. 5) has a deterministic nature. Moreover, the simplest situation is considered where the number of domains, $M(\infty)$, is independent of KP, PLT or actin concentration C_a, i.e. $P(\tau)$ is equal to the Dirac function, $\delta(0)$. Also, it is assumed that the equilibrium radius of a domain, $r(\infty)$, significantly exceeds the membrane thickness, and it is the same for all the domains. If so, according to the general representations of thermodynamics (Rusanov 1967), some balance of the forces applied to the domain perimeter must exist, as follows:

$$\sigma_m = \sigma_s + \gamma/r(\infty) \tag{6}$$

where σ_m and σ_s are the interfacial tensions that act in the regions of the bilayer not containing the domains and within the latter, respectively; γ is the so-called linear tension related to the domain curvature, $1/r(\infty)$. It is reasonable that under non-equilibrium conditions (i.e. $r \neq r(\infty)$), the radius growth rate of a separate domain, $dr(t)/dt$, should be proportional to a deviation of the force acting along its perimeter, $\gamma/r(t)$, from its equilibrium value, $\gamma/r(\infty)$:

$$\mu dr(t)/dt = \gamma[1/r(t) - 1/r(\infty)] \tag{7}$$

where μ is a parameter similar to the surface viscosity (Krotov and Malev 1979; Malev and Matveeva 1981, 1983). Remarks related to the use of Eq. 7 are contained in the appendix. Here, it is indicated that Eq. 7 seems to be valid if the domain radius growth results from absorption of adsorbed particles into the domain. In the case of their inclusion into a domain from the aqueous solution, Eq. 7 should be replaced by the following one:

$$\mu_0 dr(t)/dt = \gamma[1 - r(t)/r(\infty)] \tag{8}$$

where μ_0 is some constant (see appendix). If the initial radius of a domain is equal to $r(0)$ solutions of Eq. 7 and 8 are

$$r(t)/r(\infty) + \ln[1 - r(t)/r(\infty)] = r(0)/r(\infty) + \ln[1 - r(0)/r(\infty)] - \gamma t/\mu r^2(\infty) \tag{7a}$$

and

$$\ln[1 - r(t)/r(\infty)] = \ln[1 - r(0)/r(\infty)] - \gamma t/\mu_0 r(\infty) \tag{8a}$$

respectively.

As it was indicated above, at the gradual addition of actin the total area occupied by domains on the membrane can be found with the help of the reduced form of Eq. 5:

$$\begin{aligned} \pi R^2(t) &= \pi R^2(\infty)[R(t)/R(\infty)]^2 \\ &= \pi AM(\infty) \int_0^t r^2(t-0)\delta(0)d\tau \\ &\equiv \pi AM(\infty) r^2(t) \int_0^t \delta(0) d\tau \equiv \pi AM(\infty) r^2(t) \\ &\equiv \pi AM(\infty) r^2(\infty)[r(t)/r(\infty)]^2 \end{aligned} \tag{9}$$

where $R^2(\infty) = AM(\infty)r^2(\infty)$. This equation in principle permits one to verify the validity of either Eq. 7a or Eq. 8a for the $R(t)$-dependence, by taking into account that $N_{ch}(t)/N_{ch}(\infty) \equiv R^2(t)/R^2(\infty)$ and $N^*_{pr} \gg N_{pr}$. Figure 7a, b show that the experimental data for G-actin and PLT satisfy formally both equations, since linear dependencies are observed in all the cases. The same uncertainty takes also place for kinetic results obtained for KP (data not shown). This is not surprising, since the compared solutions (i.e. Eqs. 7a, 8a) differ from one another only at small $r(t)/r(\infty)$, but are nearly the same at greater values of the ratio. Thus, the verifications performed turn out insufficient to discriminate the above mechanisms. However, a more specified conclusion about the mechanism responsible for the domain growth can be obtained from analysis of the slopes of the obtained kinetic curves (curves (1) and (2) of Fig. 7a, b) on concentration C_a. In the case of G-actin, the slopes of curve (1) and (2) (i.e. parameters $\gamma/\mu r^2(\infty)$ and $\gamma/\mu_0 r(\theta)$, correspondingly) are increasing functions of concentration C_a (see inset to Fig. 7a), but unlike the case of actin, the slopes of curves for KP and PLT do not depend on C_a (in case of PLT, see Fig. 7b). The fact of observing the dependence of the slope on C_a is in favor of the validity of Eq. 8a for actin (its inclusion into a domain proceeds from the aqueous solution), while the absence of such dependence on the KP- and PLT-concentration most likely results from the domain formation from adsorbed polymer molecules (see Appendix).

Thus, in the first approximation, the developed representations are consistent for the influences of different compounds (actin, KP, and PLT) on the channel-forming activity of SRE, though the concrete mechanisms of their action are slightly different. One can, therefore, conclude that the obtained results support the hypothesis on the formation of regions in *cis*-monolayer, which possess an increased "affinity" to precursors of SRE-channels. If the substances in question and SRE are introduced in opposite sides of a lipid bilayer, this increased "affinity" is revealed as an increase in the SRE channel-forming activity.

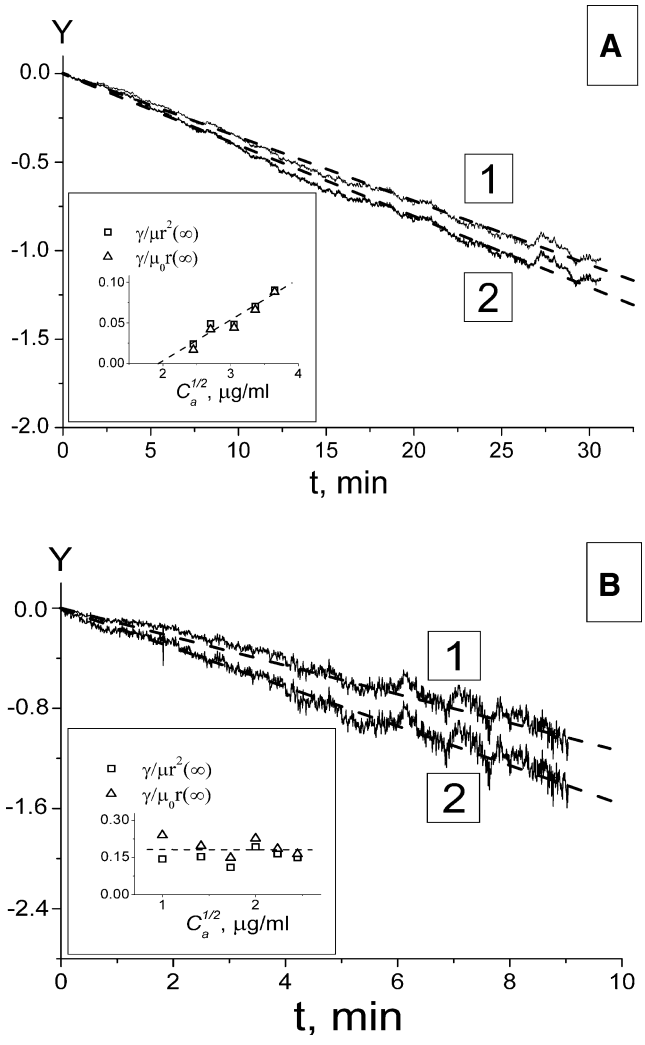

Fig. 7 Treatment of experimental $N_{ch}(t)$-curves according to Eq. 7a (1) and Eq. 8a (2) in the case of G-actin- (**a**) or PLT-addition (**b**) at the *trans*-side of the membrane. Y axis is either: $\sqrt{N_{ch}(t)/N_{ch}(\infty)} + \ln[1 - \sqrt{N_{ch}(t)/N_{ch}(\infty)}] - (\sqrt{N_{ch}(0)/N_{ch}(\infty)} + \ln[1 - \sqrt{N_{ch}(0)/N_{ch}(\infty)}])$ (curve 1) or $\ln[1 - \sqrt{N_{ch}(t)/N_{ch}(\infty)}] - \ln[1 - \sqrt{N_{ch}(0)/N_{ch}(\infty)}]$ (curve 2). Experimental conditions: DOPS/DOPE bilayer bathed in 0.1 M NaCl, pH 6. The applied voltage was 50 mV. G-actin and PLT concentrations were 9.3 and 3 μg/ml, correspondingly. Inset: dependence of $\gamma/\mu\ r^2(\infty)$ (*open square*) and $\gamma/\mu_0 r(\infty)$ (*open triangle*) on the G-actin (**a**) or PLT concentration (**b**) at the *trans*-side of the membrane

Conclusions

1. Actin promotes SRE channel-forming activity in lipid bilayers when it is added to the membrane side opposite to the SRE application. No effect is observed when actin and SRE are added to the same side of a membrane.
2. Cooperative adsorption of actin molecules on a bilayer takes place due to hydrophobic interactions and yields the formation of actin domains on the membrane surface.
3. A theoretical model based on the obtained results shows that the actin domains are responsible for the observed increase in the SRE channel-forming activity.

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Appendix

It is assumed that domain growth is a slow process that results from changes in the surface energy of a bilayer, $\Sigma(t)$, with redistribution between areas $AM(\infty)S_s$ and S_m (domain occupied and unoccupied, respectively). The energy gained under such redistribution should be spent on a dissipative process of inclusion of actin- KP-or PLT-species into the domains. In other words, the sum of the surface and dissipated energies, W(t), is equal to zero at any time t of the process, as follows:

$$\Sigma(t) + W(t) = 0 \tag{10}$$

This condition of the energy balance was first applied by Deryaguin to the consideration of capillary impregnation of grounds (Deryaguin 1946) and also to the cases of capillary rising, spreading of lenses, and black spot growth on lipid membranes (Malev and Matveeva 1981, 1983; Malev and Gribanova 1983), i.e. the processes of wetting, which are similar to what is being considered here. The surface energy of the membrane, containing $AM(\infty)$ domains of the same surface equal to S_s (see above) and $S_m = A - AM(\infty)S_s$ of unmodified surface, can be represented as follows:

$$\Sigma(t) = \int_0^t [\sigma_m dS_m/d\tau] + \sigma_s AM(\infty) dS_s/d\tau] d\tau + 2\pi AM(\infty)\gamma \int_0^t [dr(\tau)/d\tau] d\tau \tag{11}$$

If the domain growth only determines the dissipated energy, it can be written in the following form

$$\begin{aligned} W(t) &= -AM(\infty)\kappa \int_0^t S_R U[(1/S_R) dN_a/d\tau] d\tau \\ &= -AM(\infty)\kappa P \int_0^t S_R [(1/S_R) dN_a/d\tau]^2 d\tau \end{aligned} \tag{12}$$

Here, $dN_a/d\tau$ is the rate of changing the number of actin, KP or PLT species in a separate domain; U is an unknown moving force of inclusion of adsorbed species into the domain (with $U = (P/S_R) dN_a/d\tau$ if deviations from equilibrium are small); P is the resistance of the

inclusion process; $S_R = S_s = \pi\, r^2(t)$ in the case of inclusion of the species from aqueous solution, but $S_R = 2\pi\, r(t)$ in the alternative case of their inclusion from an adsorbed state (see above); κ is a factor of proportionality between the heat dissipated from a separate domain for unit time and the power $S_R U[(1/S_R)\mathrm{d}N_a/\mathrm{d}\tau]$ of the inclusion process. Substituting Eqs. 11, 12 into Eq. 10 and then differentiating the obtained equation with respect to time t, one obtains the following result:

$$\sigma_m \mathrm{d}S_m/\mathrm{d}t + \sigma_s AM(\infty)\mathrm{d}S_s/\mathrm{d}t + 2\pi AM(\infty)\gamma \mathrm{d}r(t)/\mathrm{d}t = AM(\infty)(\kappa P/S_R)[\mathrm{d}N_a/\mathrm{d}t]^2 \quad (13)$$

If area a_0 occupied by an adsorbed particle is independent of the domain radius $r(t)$, $\mathrm{d}N_a/\mathrm{d}t = (1/a_0)\mathrm{d}S_s/\mathrm{d}t = [2\pi\, r(t)/a_0]\mathrm{d}r(t)/\mathrm{d}t$. As a result, Eq. 13 reduces to

$$\sigma_s - \sigma_m + \gamma/r(t) = \mu \mathrm{d}r(t)/\mathrm{d}t \quad \text{at } S_R = 2\pi r(t),\ (\mu = \kappa P/a_0^2) \quad (14)$$

and

$$\sigma_s - \sigma_m + \gamma/r(t) = [\mu_0/r(t)]\mathrm{d}r(t)/\mathrm{d}(t) \quad \text{at } S_R = r^2(t),\ (\mu_0 = 2\kappa P/a_0^2) \quad (15)$$

In the general case, all tensions included in the above equations are dependent on the concentrations of adsorbing particles in the unmodified regions of the *trans*-side of the membrane. This is not the case if the rate of adsorption (on unmodified regions of the bilayer) is higher than that of domain growth. If so, the difference $(\sigma_m - \sigma_s)$ can be replaced with $\gamma/r(\infty)$ in accordance with the equilibrium condition given by Eq. 6. As a result, Eqs. 14 and 15 take the forms of Eqs. 7 and 8, respectively. Note that, in the partial case of the black spot growth on colored lipid membranes (Malev and Matveeva 1983), the rate of the domain growth, $\mathrm{d}r(t)/\mathrm{d}t$, is constant and proportional to $(\sigma_m - \sigma_s)$, since the value of this difference is high enough and cannot be compensated by linear tension γ at any macroscopic value of the spot radius, $r(\infty)$.

In Eq. 11 it is assumed that possible gradients in the concentration of separate adsorbing particles are absent within unmodified regions of *trans*-side of the membrane. This assumption is correct for absorption of particles into domains from aqueous solution, but may be invalid for inclusion from their adsorbed state. Eq. 11 can be written in a form that accounts for gradients of the adsorbing species, but a problem arises with such attempts. Unlike the previous considerations, an equation for the domain radius increase must be put in a concrete form, since resistance P (see the paragraph before Eq. 13) of the inclusion process should depend on the concentration of adsorbed particles at distance $r(t)$ (i.e. the domain radius) from the domain centre. Parameters $\gamma/\mu\ r^2(\infty) = \gamma\ a_0^2/\kappa\ Pr^2(\infty)$ and $\gamma/\mu_0 r(\infty) = \gamma\ a_0^2/2\kappa\ Pr(\infty)$ of Eqs. 7a and 8a, respectively, might be dependent on the concentration of adsorbing particles, since the equilibrium radius $r(\infty)$ must obviously increase with increasing concentration C_a. In particular, $r^2(\infty)$ should be proportional to concentration C_a according to the phase theory of micelle formation (domain formation in our case). On the other hand, resistance P included in the above parameters is, most likely, in reverse proportion to concentration C_a, as is characteristic for heterogeneous process. If so, $\gamma\ a_0^2/\kappa\ Pr^2(\infty)$ turns out independent of concentration C_a, while $\gamma\ a_0^2/2\kappa\ Pr(\infty) \sim C_a^{1/2}$. Taking into account the observed increase in the kinetic dependence of $\ln [1 - r(t)/r(\infty)]$ vs. time t with increasing C_a (inset to Fig. 7a), it is speculated that a direct inclusion of actin molecules into domains from aqueous solution is more probable than the mechanism with participation of adsorbed actin species.

References

Bard AJ, Faulkner LR (2001) Electrochemical Methods: Fundamentals and Applications, 2nd edn. Wiley, New York

Bender CL, Alarcon-Chaidez F, Gross DC (1999) Pseudomonas syringae phytotoxins: mode of action, regulation and biosynthesis by peptide and polyketide synthetases. Microbiol Mol Biol Rev 63:266–292

Berdiev BK, Prat AG, Cantiello HF, Ausiello DA, Fuller CM, Jovov B, Benos DJ, Ismailov II (1996) Regulation of epithelial sodium channels by short actin filaments. J Biol Chem 271:17704–17710

Berdiev BK, Latorre R, Benos DJ, Ismailov II (2001) Actin modifies Ca^{2+} block of epithelial Na^+ channels in planar lipid bilayers. Biophys J 80:2176–2186

Bessonov AN, Gurnev FA, Kuznetsova IM, Takemoto JY, Turoverov KK, Malev VV, Schagina LV (2004) Interaction between filamentous actin and lipid bilayer causes the increase of syringomycin E channel-forming activity. Tsitologiia (in Russian) 46:628–633

Bidwai AP, Zhang L, Bachmann RC, Takemoto JY (1987) Mechanism of action of *Pseudomonas syringae* phytotoxin, syringomycin: stimulation of red beet plasma membrane ATPase activity. Plant Physiol 83:39–43

Bouchard M, Pare C, Dutasta JP, Chauvet JP, Gicquaud C, Auger M (1998) Interaction between G-actin and various types of liposomes: A 19F, 31P, and 2H nuclear magnetic resonance study. Biochemistry 37:3149–3155

Buijs J, Ramstrom M, Danfelter M, Larsericsdotter H, Hakansson P, Oscarsson S (2003) Localized changes in the structural stability of myoglobin upon adsorption onto silica particles, as studied with hydrogen/deuterium exchange mass spectrometry. J Colloid Interface Sci 263:441–448

Deryaguin BV (1946) Determination of porous body specific surface due to the rate of capillary impregnation. Kolloidny Zhournal (in Russian) 8:27–30

Eskesen K, Kristensen BI, Jorgensen AJ, Kristensen P, Bennekou P (2001) Calcium-dependent association of annexins with lipid bilayers modifies gramicidin A channel parameters. Eur Biophys J 30:27–33

Feigin AM, Takemoto JY, Wangspa R, Teeter JH, Brand JG (1996) Properties of voltage-gated ion channels formed by syringomycin E in planar lipid bilayers. J Membr Biol 149:41–47

Feigin AM, Schagina LV, Takemoto JY, Teeter JH, Brand JG (1997) The effect of sterols on the sensitivity of membranes to the channel-forming antifungal antibiotic, syringomycin E. Biochim Biophys Acta 1324:102–110

Gicquaud C (1995) Does actin bind to membrane lipids under conditions compatible with those existing in vivo? Biochem Biophys Res Commun 208:1154–1158

Goldmann WH, Teodoridis JM, Sharma CP, Alonso JL, Isenberg G (1999) Fragments from alpha-actinin insert into reconstituted lipid bilayers. Biochem Biophys Res Commun 264:225–229

Grigoriev PA, Tarahovsky YuS, Pavlik LL, Udaltsov SN, Moshkov DA (2000) Study of F-actin interaction with planar and liposomal bilayer phospholipid membranes. IUBMB Life 50:227–233

Gurnev PhA, Kaulin YuA, Takemoto JY, Schagina LV, Malev VV (2002) Effects of charges and dipole moments of membrane lipids on gating properties of ion channels induced by syringomycin E. Biol Membr (in Russian) 19: 244–250

Gurnev PhA, Bessonov AN, Kuznetsova IM, Malev VV, Pershina VP, Pinaev GP, Turoverov KK, Takemoto JY, Tikhomirova AV, Schagina LV (2003) Effects of actin and some polyions on channel-forming a ctivity of syringomycin E in bilayer lipid membranes. Biol Membrany (in Russian) 20:421–428

Han X, Li G, Li G, Lin K (1997) Interactions between smooth muscle alpha-actinin and lipid bilayers. Biochemistry 36:10364–10371

Isenberg G, Niggli V (1998) Interaction of cytoskeletal proteins with membrane lipids. Int Rev Cytol 178:73–125

Ismailov II, Berdiev BK, Shlyonsky VG, Fuller CM, Prat AG, Jovov B, Cantiello HF, Ausiello DA, Benos DJ (1997) Role of actin in regulation of epithelial sodium channels by CFTR. Am J Physiol 272:1077–1086

Jacobson GR, Rosenbusch JP (1976) ATP binding to a protease-resistant core of actin. Proc Natl Acad Sci USA 73:2742–2746

Kaulin YA, Schagina LV, Bezrukov SM, Malev VV, Feigin AM, Takemoto JY, Teeter JH, Brand JG (1998) Cluster organization of ion channels formed by the antibiotic syringomycin E in bilayer lipid membranes. Biophys J 74:2918–25

Konig T, Stipani I, Horvath I, Palmieri F (1982) Inhibition of mitochondrial substrate anion translocators by a synthetic amphipathic polyanion. J Bioenerg Biomembr 14:297–305

Krotov VV, Malev VV (1979) Rheology of free liquid films with surfactants. Kolloidnyi Zhournal (in Russian) 41:49–53

Malev VV, Gribanova EV (1983) Wetting resistance in kinetics of capillary rise of liquids. Dokl Akad Nauk SSSR (in Russian) 272:413–416

Malev VV, Matveeva AI (1981) Kinetics of lenses spreading on free liquid films. Dokl Akad Nauk SSSR (in Russian) 261:685–689

Malev VV, Matveeva AI (1983) Kinetics of bilayer lipid membrane formation. Biofizika (in Russian) 28:50–55

Malev VV, Kaulin YuA, Bezrukov SM, Gurnev PhA, Takemoto JY, Schagina LV (2001) Kinetics of opening and closure of syringomycin E channels formed in lipid bilayers. Membr Cell Biol 14:813–829

Malev VV, Schagina LV, Gurnev PA, Takemoto JY, Nestorovich EM, Bezrukov SM (2002) Syringomycin E channel: a lipidic pore stabilized by lipopeptide? Biophys J 82:1985–1994

Maltseva EA, Antonenko YuN, Melik-Nubarov NS, Yaguzhinsky LS (2002) Effect of synthetic amphiphilic polyanions on the ion permeation through planar bilayer lipid membrane. Biol Membr (in Russian) 19:347–350

Montall M, Muller P (1972) Formation of bimolecular membranes from lipid monolayers and study of their electrical properties. Proc Natl Acad Sci USA 65:3561–3566

Mornet D, Ue K (1984) Proteolysis and structure of skeletal muscle actin. Proc Natl Acad Sci USA 81:3680–3684

Niggli V (2001) Structural properties of lipid-binding sites in cytoskeletal proteins. Trends Biochem Sci 26:604–611

Niggli V, Dimitrov DP, Brunner J, Burger MM (1986) Interaction of the cytoskeletal component vinculin with bilayer structures analyzed with a photoactivatable phospholipid. J Biol Chem 261:6912–6918

Pardee JD, Spudich JA (1982) Purification of muscle actin. Methods Enzymol 85:164–181

Rees MK, Young M (1967) Studies on the isolation and molecular properties of homogeneous globular actin. Evidence for a single polypeptide chain structure. J Biol Chem 242:4449–4458

Rusanov AI (1967) Phase equilibria and surface phenomena. Khimia Leningrad (in Russian)

Schagina LV, Kaulin YA, Feigin AM, Takemoto JY, Brand JG, Malev VV (1998) Properties of ionic channels formed by the antibiotic syringomycin E in lipid bilayers: dependence on the electrolyte concentration in the bathing solution. Membr Cell Biol 12:537–555

Schagina LV, Gurnev PhA, Takemoto JY, Malev VV (2003) Effective gating charge of ion channels induced by toxin syringomycin E in lipid bilayers. Bioelectrochem 60:21–27

Sheterline P, Clayton J, Sparrow JC (1999) Actin, 4th edn. University Press Oxford

Shinoda K, Nakagawa T, Tamamushi BI, Isemura T (1963) Colloidal surfactants: some physicochemical properties. Academic, New York

St-Onge D, Gicquaud C (1989) Evidence of direct interaction between actin and membrane lipids. Biochem Cell Biol 67:297–300

St-Onge D, Gicquaud C (1990) Research on the mechanism of interaction between actin and membrane lipids. Biochem Biophys Res Commun 167:40–47

Suchyna TM, Tape SE, Koeppe RE II, Andersen OS, Sachs F, Gottlieb PA (2004) Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers. Nature 430:235–240

Tsunoda T, Imura T, Kadota M, Yamazaki T, Yamauchi H, Kwon KO, Yokoyama S, Sakai H, Abe M (2001) Effects of lysozyme and bovine serum albumin on membrane characteristics of dipalmitoylphosphatidylglycerol liposomes. Colloids Surf B Biointerfaces 20:155–163

Turoverov KK, Khaitlina SY, Pinaev GP (1976) Ultra-violet fluorescence of actin. Determination of native actin content in actin preparations. FEBS Lett 62:4–7

Xu X, Forbes JG, Colombini M (2001) Actin modulates the gating of *Neurospora crassa* VDAC. J Membr Biol 180:73–81