Oscillations in the Lateral Pressure of Lipid Monolayers Induced by Nonlinear Chemical Dynamics of the Second Messengers MARCKS and Protein Kinase C

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ABSTRACT   The binding of the MARCKS peptide to the lipid monolayer containing PIP$_2$ increases the lateral pressure of the monolayer. The unbinding dynamics modulated by protein kinase C leads to oscillations in lateral pressure of lipid monolayers. These periodic dynamics can be attributed to changes in the crystalline lipid domain size. We have developed a mathematical model to explain these observations based on the changes in the physical structure of the monolayer by the translocation of MARCKS peptide. The model indicates that changes in lipid domain size drives these oscillations. The model is extended to an open system that sustains chemical oscillations.

INTRODUCTION

Cellular membranes are formed by different types of proteins dispersed in a lipid bilayer. Proteins and lipids are not randomly distributed in the membrane but they associate according to their hydrophobic and electrical properties (1). Spatial organization of lipids and proteins in membranes is still a puzzling problem. Lipid mixtures can assemble in domainlike, inhomogeneous structures (2), and self-organization among different proteins processes can give rise to the formation of traveling waves in lipid bilayers mimicking the membranes of bacteria (3). There is an increasing interest for the nonequilibrium self-organization of spatiotemporal structures in controlled in vitro experiments to understand similar behavior in living cells.

Phosphatidylinositol 4,5-biphosphate (PIP$_2$) is a phospholipid component of the cell membrane. This phospholipid performs an important function in membranes for signaling. Its hydrolysis by phospholipase C generates two second messengers: inositol 1,4,5-triphosphate and diacylglycerol. To avoid the onset of the process, PIP$_2$ is sequestered by myristoylated alanine-rich C kinase substrate (MARCKS) (4). MARCKS is an unfolded protein that has multiple functions and is related to the regulation of cytoskeletal dynamics (5), phagocytosis (6), and exocytosis (7). The effector domain of MARCKS interacts electrostatically with PIP$_2$, causes the binding of the protein to the membrane (8), and inhibits the hydrolysis of the phospholipid. The action of protein kinase C (PKC) reverses this process because phosphorylated MARCKS no longer binds to PIP$_2$ in the membranes and diffuses in the cytosol (9). PKC is activated and translocated to the membrane by calcium, diacylglycerol, and phospholipids (10). It is also known that PIP$_2$ enhances the translocation of PKC (11–13).

The regulating binding and unbinding cycle of MARCKS constitutes a reaction-diffusion system that allows us to study the impact of pattern formation on signal transduction (14–16). Furthermore, PKC oscillations produced by calcium oscillations have been observed (17) and the possible interactions and feedbacks on the calcium dynamics have been discussed (18).

A well-suited method for studying pattern formation of lipids within an interface is the Langmuir monolayer technique. In this reduced scheme, the monolayer represents the inner layer of the membrane and the Langmuir subphase represents the cell cytosol. The translocations of MARCKS and PKC between the monolayer and the subphase and the phosphorylation of MARCKS by PKC in the monolayer are sketched in Fig. 1. The reduced number of ingredients in the Langmuir monolayer permits the description of the observations by a mathematical kinetic model.

Here, we study the binding of MARCKS peptides (consisting on the effector domain of MARCKS proteins) to monolayers containing PIP$_2$. The interaction among phospholipids, MARCKS peptides, and PKC produces a cyclic translocation of the peptides in the monolayer. We interpret the observations and propose a mechanism for the oscillations. The mathematical formulation of such a mechanism as a reaction-diffusion model permits us to obtain numerical results and facilitates comparison with the experimental observations. This model can be further extended to open conditions, where several biological concentrations are continuously submitted. The extended model predicts coherent oscillations.

MATERIALS AND METHODS

Experiment

To study the interaction between MARCKS and PIP$_2$, we have chosen an electrostatically neutral lipid as matrix for the charged lipid. According to the measurements at the air/water interface, saturated lipids with the
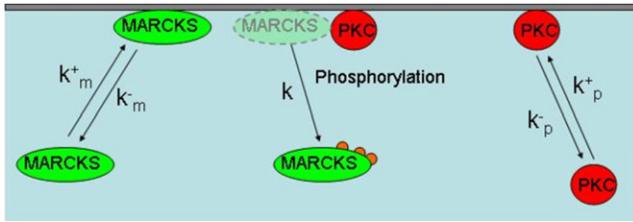

FIGURE 1 Sketch of the attachment-detachment mechanism of MARCKS and PKC and of the phosphorylation of MARCKS by PKC.

same lipid chain length are employed to achieve a quite stable configuration. The long size of the lipid chains precludes any perturbation by Marangoni effects (19). Thus, the monolayer was composed by DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) and PIP$_2$ (1,2-dipalmitoyl-phosphatidylinositol 4,5-diphosphate), both from Sigma-Aldrich (St. Louis, MO).

The lipid solutions were mixed to the desired PIP$_2$ content before spreading. The amount of PIP$_2$ in the lipid system was always 10 mol %. The mixed lipid solution was spread on a subphase, consisting of 100 mM NaCl, 10 mM HEPES, 1 mM CaCl$_2$, and 5 mM adenosine triphosphate (to provide the phosphate for phosphorylation by PKC), dissolved in Millipore water (Billerica, MA), adjusted to pH 7.4. Finally, 0.1 μM MARCKS (151–175) peptide was added to the subphase. This peptide sequence, the so-called effector domain, is responsible for the electrostatic interaction with the membrane interface (4) and substitutes the MARCKS protein in our experiment. MARCKS (151–175) was obtained as lyophilized powder from AnaSpec (San Jose, CA) and dissolved in Millipore water.

The measurements are carried out with a homemade film balance and a KSV Minimicro LB system (Helsinki, Finland). The Minimicro film balance trough is equipped with a special port for the PKC injection into the subphase. In this way, we avoid a disruption of the monolayer due to injection. PKC containing the α-, β-, and γ-isotypes was obtained from Biomol International (Plymouth Meeting, PA).

Model

A complete model of the system would contain variables defined in a three-dimensional subphase as well as variables defined at a two-dimensional interface. However, the experimental measurements record the lateral pressure, an integral quantity that depends on the whole monolayer. Due to the large size of the monolayer (6.25 cm^2) and to simplify the model, we assume that the monolayer is effectively in a spatially homogeneous state and we neglect the spatial details of the attachment-detachment processes. Because we neglect spatial changes in the monolayer, we can restrict the modeling to the computation of one-dimensional profiles along the z-coordinate describing the direction orthogonal to the membrane plane.

To derive the model, we divide the system into two compartments: a narrow layer, where all the reactions take place ($0<z<\ell$); and a passive subphase ($\ell<z<L$), where ℓ is the size of this narrow layer (16) and L the vertical size of the system. The details of the reaction processes in this narrow layer will be fixed in the next sections. Three chemical species: MARCKS peptides (M), PKC enzymes (P), and phosphorylated MARCKS peptides (M_P) diffuse in the subphase ($\ell<z<L$),

$$
\begin{aligned}
\partial_t M(z,t) &= D_M \partial_z^2 M(z,t), \\
\partial_t P(z,t) &= D_P \partial_z^2 P(z,t), \\
\partial_t M_p(z,t) &= D_M \partial_z^2 M_p(z,t),
\end{aligned}
\tag{1}
$$

where D_M and D_P are the diffusion coefficients of MARCKS and PKC, respectively. The values of the diffusion constants are estimated from the

molecular weight (20). The reactions occur only near the monolayer ($0<z<\ell$),

$$
\begin{aligned}
\partial_t M(z,t) &= -R_M + D_M \partial_z^2 M(z,t), \\
\partial_t P(z,t) &= -R_P + D_P \partial_z^2 P(z,t), \\
\partial_t M_p(z,t) &= R_{MP} + D_M \partial_z^2 M_p(z,t),
\end{aligned}
\tag{2}
$$

where the terms R_i correspond to the nonlinear reaction rates and attachment-detachment processes and in general may depend on the MARCKS and PKC concentrations in the subphase and at the monolayer, and on the state of the monolayer (see below).

Formally, we may define the concentration of monolayer-bound proteins as

$$
\partial_t \tilde{m}(z,t) = R_M - R_{MP} + D_{\tilde{m}} \nabla^2 \tilde{m}(z,t).
$$

We neglect the structure in the narrow layer near the membrane and define the average

$$
m(t) = (1/\ell) \int_0^\ell \tilde{m}(z,t)dz.
$$

The contribution of the diffusion is zero and we approximate the reaction rates with

$$
(1/\ell) \int_0^\ell R_i dz \approx (1/\ell)R_i \ell = R_i,
$$

which leads to Eq. 3. Equivalent arguments can be used to obtain equations for the average quantities $p(t)$ and $\theta(t)$.

The corresponding equations for the averaged concentration of MARCKS peptides (m) and PKC enzymes (p) at the monolayer are obtained from an approximation and read

$$
\dot{m}(t) = R_M - R_{MP},
\tag{3}
$$

$$
\dot{p}(t) = R_P.
\tag{4}
$$

To model the interaction between the monolayer and the subphase, we consider a narrow layer from which proteins bind and into which proteins are released from the monolayer. This layer is diffusively coupled to the subphase.

We define the integral quantities

$$
\phi_M = L^{-1} \int_0^L M dz \text{ and } \phi_{M_p} = L^{-1} \int_0^L M_p dz,
$$

which represent the total amount of free and phosphorylated MARCKS peptides in the subphase, respectively. Equivalently we define the total amount of PKC in the subphase as

$$
\phi_P = L^{-1} \int_0^L P dz.
$$

The total amount of peptides

$$
\phi_{M,T} = m + \phi_M
$$

and of enzymes

$$
\phi_{P,T} = p + \phi_P
$$

are conserved quantities, and hence are fixed parameters in the model.

Finally, we also need to consider a variable θ to account for the structure of the monolayer. The value of θ is related with the accessible quantity of

PIP_2. We introduce this variable to account for the change of the monolayer structure upon binding of the peptides, which in turn affects the attachment rates of the peptides. We use a simple phenomenological equation to describe the dependence of the variable θ on the monolayer-bound MARCKS:

$$\dot{\theta}(t) \; = \; R_\theta. \qquad (5)$$

We employ a finite difference method in one dimension to perform numerical simulations of the diffusion of the species. For the boundaries of our one-dimensional model system (bottom and top of the container), we impose no-flux conditions. The size of the narrow layer in the numerical simulations is small and we choose $\ell = \Delta z$ to avoid any type of structure near the monolayer. The total amount of peptides is kept constant. It is fixed by the initial condition.

RESULTS

Translocation of MARCKS

Experiments

First, we perform experiments without the introduction of PKC in two different versions. Either the monolayer of the lipid mixture is compressed from a null lateral pressure to 10 mN/m $\pm$ 0.5 mN/m or the lipid mixture is spread on an initial pressure of 20 mN/m $\pm$ 0.5 mN/m. A difference in the size of the liquid condensed lipid domains is observed depending on the manner of preparation. The ordered lipid domains in a slowly compressed monolayer are larger and better developed with respect to a monolayer, and directly spread out on a certain pressure.

We probe both variants to exclude a dependency on the lateral organization of the monolayer. At a lateral pressure of 10 mN/m, the monolayer is already above the main phase transition in a liquid condensed state with remnants of disordered loosely packed regions, and the interaction between MARCKS peptide and the monolayer can still be visualized by fluorescence microscopy. Above 10 mN/m, the dye distribution disappears with increase in lateral pressure and a visual observation is no longer possible. Nevertheless, the remnants of disordered phase are preserved (22). Here, the interaction can be only recorded indirectly through lateral pressure measurements.

Initially, there is an homogeneous distribution of the MARCKS peptide in the subphase, because of its good solubility in water. Control experiments do not show any activity of the peptide at the bare buffer/air interface. When the monolayer is prepared on the subphase, the peptide begins to translocate to the monolayer. Attachment of MARCKS peptide to the monolayer causes the lateral pressure to increase monotonically due to a partial penetration of the peptide into the monolayer (23,24). This increase of the lateral pressure is effectively only caused by the effector domain of MARCKS.

Experiments with EDTA in the subphase instead of $CaCl_2$ have shown a very similar behavior. In both experiments, the increase of the lateral pressure is completely the same—no
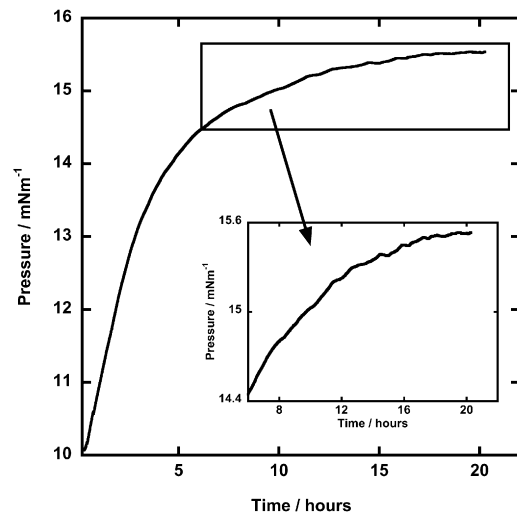

FIGURE 2   Increase of lateral pressure in the compressed monolayer due to attachment of MARCKS peptide.

dependence on the lateral organization is detectable. We assume the lateral pressure is proportional to the concentration of MARCKS peptide attached to the monolayer. The increase of the lateral pressure ends with the peptide saturation of the monolayer (see Fig. 2). This process is slow and governed by a diffusion-limited attachment to the monolayer. The characteristic diffusion time of the peptide through the subphase to the monolayer is estimated to be approximately hours because of an $L = 5$ mm subphase thickness. The saturated monolayer remains stable over a long period of time (see *inset* in Fig. 2) and only slight fluctuations are observed.

The attachment of the MARCKS peptide to the monolayer produces also a change in the monolayer structure. The lipid mixture builds a monolayer, formed by solid DPPC domains surrounded by liquid domains rich in PIP_2 (23). The lateral organization of the monolayer will depend on the presence of bound peptide at the monolayer and will continuously change with the attachment of peptide. In Fig. 3, in two different experimental images corresponding to the structure of the compressed monolayer is shown the difference between the monolayer without and with
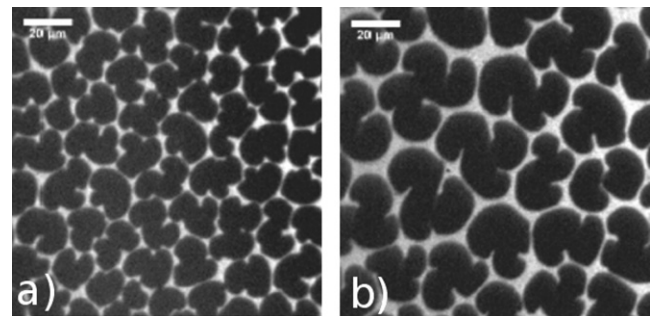

FIGURE 3   Fluorescence micrographs of (*a*) the initial state and (*b*) after attachment of MARCKS at the monolayer (24).

MARCKS peptide, corresponding to Fig. 3, a and b, respectively. The presence of MARCKS peptide at the monolayer causes larger solid domains and simultaneously larger areas of liquid domains. This is explained by the enrichment of PIP_2 in the disordered phase, whereby the interaction with MARCKS peptide consequently occurs in this phase. The images, shown in Fig. 3, correspond to a lateral pressure of $\pi = 15$ mN/m. However, regions of disordered phase persist at higher lateral pressure (23).

The final pressure of the monolayer depends on the initial concentration of MARCKS peptide in the subphase. The change of the pressure is small for low concentrations but it saturates for larger concentrations. For initial concentrations above 25 nM, the final lateral pressure of the monolayer is always around the same value. This concentration defines the monolayer saturation concentration.

Model

Corresponding to the experimental setup, we consider a closed system with an initial condition far from equilibrium. Such an initial condition drives a transient dynamics of the system approaching an equilibrium state after sufficiently long time. The peptides diffuse through the subphase. They translocate to the monolayer and from the monolayer to the subphase near the monolayer. The concentration M of the peptide in the subphase is described by

$$\partial_t M(z,t) = D_M \partial_z^2 M(z,t). \qquad (6)$$

The equations describing translocation dynamics of the peptide close to the monolayer reads

$$\partial_t M(z,t) = -[1 - m(t)]k_m^+(\theta)M(z,t) + k_m^- m(t) + D_M \partial_z^2 M(z,t), \qquad (7)$$

and the corresponding equations at the monolayer,

$$\dot{m}(t) = [1 - m(t)]k_m^+(\theta)M(z,t) - k_m^- m(t),$$
$$\dot{\theta}(t) = k_\theta[m(t) - \theta(t)], \qquad (8)$$

where the variables M and m correspond to the fractions of MARCKS in the subphase and the fraction of MARCKS bound to the monolayer, respectively. The attachment and detachment of the peptide to the monolayer is accounted by $k_m^+(\theta)$ and $k_m^-(\theta)$.

The variable m is a nondimensional quantity ($0 < m < 1$) describing the coverage of MARCKS on the lipid monolayer, where $m = 1$ corresponds to maximum coverage. The term $(1 - m)$ precludes the attachment of peptides after the saturation of the monolayer. The variable M is a nondimensional quantity obtained from the renormalization by the volume saturation concentration of MARCKS. We recall that the quantity

$$\phi_M = L^{-1} \int_0^L M dz$$

is the total amount of MARCKS peptides in the subphase divided by the monolayer saturation value.

The variable θ corresponds to the state of the monolayer (i.e., $\theta = 0$ corresponds to small domains as in Fig. 3 a and $\theta = 1$ to large domains; see in Fig. 3 b) and its phenomenological equation tries to describe the change of the domain size and the properties of the monolayer with the binding of MARCKS.

The function $k_m^+(\theta)$ is nonlinear, and accounts for the dependence of the attachment rate of MARCKS on the state of the monolayer (θ). The explicit forms of the function employed here is a Hill function

$$k_m^+(\theta) = k_m^+ \theta^2 / (\theta^2 + K_1^2).$$

If k_θ is large, we can adiabatically eliminate this variable using $\theta = m$ in the function $k_m^+(\theta)$ and obtain finally a Hill function of the concentration m. A similar dependence on the concentration has been previously observed in experiments of binding of MARCKS to vesicles (25).

Simulations

We integrate Eqs. 6–8 to study the dynamics of MARCKS peptides interacting with the monolayer. Based on an initially homogeneous distribution M of MARCKS in the subphase, there is a monotonous increase of the concentration of monolayer-bound peptides m (see Fig. 4 a). It implies an increase of the monolayer pressure due to the binding of MARCKS because the lateral pressure is assumed to be proportional to m. The translocation to the monolayer produces a decrease of concentration in the subphase region near the membrane. This, in turn, induces a concentration gradient of free peptide in the subphase, which induces the transport of more peptide to the monolayer. If the system remains undisturbed, the peptide accumulates at the monolayer until saturation is reached (see Fig. 4). The simulation
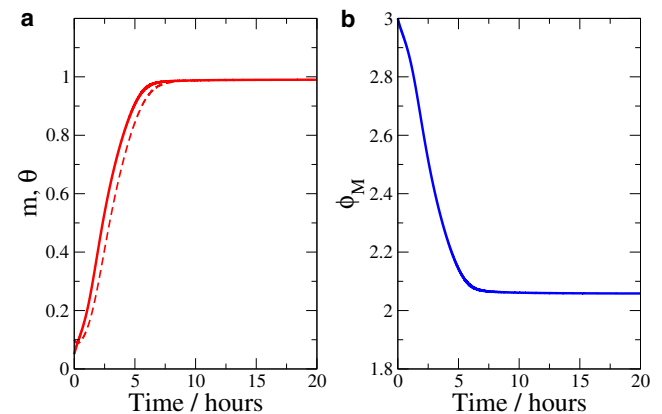

FIGURE 4 Temporal evolution of m (*solid line*) and θ (*dashed line*) at the monolayer (*a*), and of the fractions ϕ_M (*solid line*) in the subphase (*b*) during a numerical simulation. Parameters of the numerical simulations: $D_M = 160$ μm²/s, $k_m^+ = 1$ s⁻¹, $K_1 = 1$, $k_m^- = 2 \times 10^{-5}$ s⁻¹, and $\phi_{M,T} = 3$.

reproduces the results observed in the experiments shown in Fig. 2. Both curves exhibit similar times (~3 h) for reaching half of the saturation pressure respective to concentration.

The total quantity of peptide is kept constant during the simulation. It implies that the increase on the concentration of monolayer-bound proteins (m) produces a reduction on the concentration of MARCKS in the subphase (ϕ_M) (see Fig. 4 b).

Phosphorylation of MARCKS by PKC

Model

The enzyme PKC diffuses in the cytosol, and phosphorylates membrane-bound MARCKS proteins in living cells (4). To study this process, we consider the effects of the introduction of PKC into the system. This enzyme has binding affinity to the monolayer where it becomes active (10). We assume an attachment-detachment process for the PKC similar to the one previously considered for MARCKS. The equations describing the mentioned processes are

$$\partial_t P(z,t) = D_P \partial_z^2 P(z,t), \qquad (9)$$

for the diffusion of PKC in the subphase,

$$\partial_t P(z,t) = -[1-m(t)][1-p(t)]k_p^+(\theta)P(z,t) \\ + k_p^- p(t) + D_P \partial_z^2 P(z,t), \qquad (10)$$

for the reactions close to the monolayer and at the monolayer, and

$$\dot{p}(t) = [1-m(t)][1-p(t)]k_p^+(\theta)P(z,t) - k_p^- p(t), \quad (11)$$

where the variable p is a nondimensional quantity ($0 < p < 1$) describing the coverage of PKC on the lipid monolayer and $p = 1$ corresponds to maximum coverage. The variable P is a nondimensional quantity obtained from a scaling of the physical concentration of PKC by a volume saturation concentration of PKC. The function $k_p^+(\theta)$ is nonlinear, and accounts for the dependence of the attachment rate of PKC on the state of the monolayer (θ), that is related to the available phospholipid concentration. The explicit form employed here is a Hill function

$$k_p^+(\theta) = k_p^+ \theta^n / (\theta^n + K_2^n)$$

with a large Hill coefficient $n = 8$. A similar dependence has been previously observed for PKC attachment to phospholipids (25,26).

The activation of PKC leads to the phosphorylation of MARCKS peptides. To account for this additional translocation process, an additional term in the dynamics of the MARCKS peptide in the membrane is employed and the model equations have to be supplemented by additional equations for the concentration of phosphorylated

MARCKS peptides M_p. The diffusion of phosphorylated MARCKS peptides in the subphase is described by

$$\partial_t M_p(z,t) = D_M \partial_z^2 M_p(z,t). \qquad (12)$$

The PKC-induced translocation and phosphorylation of MARCKS follows the rate equations

$$\partial_t M_p(z,t) = km(t)p(t) + D_M \partial_z^2 M_p(z,t) \qquad (13)$$

close to the monolayer and

$$\dot{m}(t) = [1-m(t)]k_m^+(\theta)M(z,t) - k_m^- m(t) - km(t)p(t) \quad (14)$$

at the monolayer. The variable M_p is a nondimensional quantity obtained from the rescaling of the concentration of phosphorylated MARCKS peptides by the volume saturation concentration. The phosphorylation process requires the presence of adenosine triphosphate, which is assumed to have a constant concentration and to be homogeneously distributed during the whole process. (Note that kinetic parameters of the equations are given in the caption of Fig. 4.) The final set of equations of the model reads

$$\begin{aligned} \partial_t M(z,t) &= D_M \partial_z^2 M(z,t), \\ \partial_t P(z,t) &= D_P \partial_z^2 P(z,t), \\ \partial_t M_p(z,t) &= D_M \partial_z^2 M_p(z,t), \end{aligned} \qquad (15)$$

for the diffusion processes in the subphase ($\ell < z < L$),

$$\begin{aligned} \partial_t M(z,t) &= -[1-m(t)]k_m^+(\theta)M(z,t) + k_m^- m(t) \\ &\quad + D_M \partial_z^2 M(z,t), \\ \partial_t P(z,t) &= -[1-m(t)][1-p(t)]k_p^+(\theta)P(z,t) \\ &\quad + k_p^- p(t) + D_P \partial_z^2 P(z,t), \\ \partial_t M_p(z,t) &= km(t)p(t) + D_M \partial_z^2 M_p(z,t), \end{aligned} \qquad (16)$$

close to the monolayer ($0 < z < \ell$) and

$$\begin{aligned} \dot{m}(t) &= [1-m(t)]k_m^+(\theta)M(z,t) - k_m^- m(t) - km(t)p(t), \\ \dot{p}(t) &= [1-m(t)](1-p(t))k_p^+(\theta)P(z,t) - k_p^- p(t), \\ \dot{\theta}(t) &= k_\theta[m(t) - \theta(t)], \end{aligned} \qquad (17)$$

at the monolayer. Capital letters describe the concentration in the subphase. This set of equations accounts for the binding dynamics of MARCKS peptide and PKC and the interaction between them.

Simulations

Fig. 5 shows the evolution of the corresponding quantities of the model when the equations are solved numerically. Initially, the concentration of monolayer-bound MARCKS peptide grows with time. However, if PKC is introduced, there are damped oscillations in the monolayer (Fig. 5 a). PKC is introduced with a strongly localized concentration distribution inside the subphase and close to the monolayer.
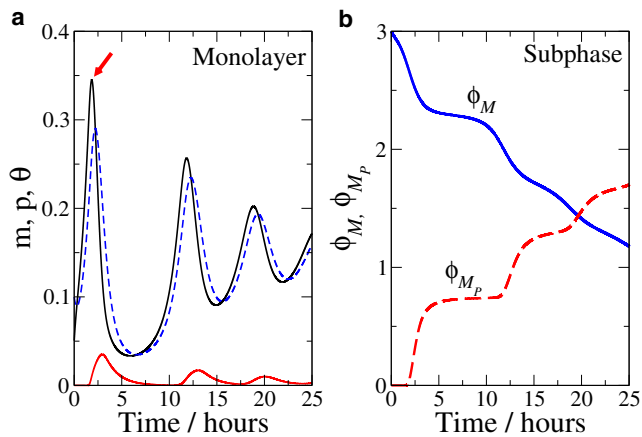
FIGURE 5 Temporal evolution of m (*dark line*), p (*line*), and θ (*dashed line*) at the monolayer (*a*), and of the fractions ϕ_M (*solid line*) and ϕ_{M_p} (*dashed line*) in the subphase (*b*) during a numerical simulation. (*Arrow*) Moment of the introduction of PKC. Parameters of the numerical simulations: $D_M = 160~\mu m^2/s$, $D_P = 60~\mu m^2/s$, $k^+{}_m = 1~s^{-1}$, $k^-{}_m = 2 \times 10^{-5}$ s^{-1}, $k^+{}_p = 0.2~s^{-1}$, $k^-{}_p = 2 \times 10^{-4}~s^{-1}$, $k = 2 \times 10^{-2}~s^{-1}$, $k_\theta = 0.0005$ s^{-1}, $K_1 = 1$, $K_2 = 0.25$, $n = 8$, $\phi_{M,T} = 3$, and $\phi_{P,T} = 0.1$.

The total concentration of PKC ($\phi_{P,T}$) is fixed for the rest of the simulation. The enzyme drastically reduces the concentration of attached MARCKS at the monolayer, due to the phosphorylation of the peptide. This detachment of MARCKS is visible in the decrease of the lateral pressure of the monolayer.

After this initial reduction, a damped oscillatory behavior of the lateral pressure in the monolayer is observed. The amplitude of the pressure oscillation decreases after some cycles because the reservoir of available unphosphorylated peptide is depleted at any time and the phosphorylated peptide remains in the subphase. There are damped oscillations in the monolayer (Fig. 5 *a*) accompanied by a decrease and an increase of ϕ_M and ϕ_{M_p}, respectively (Fig. 5 *b*). In Fig. 5, we employ a specific set of parameter values. The oscillations are, however, robust for a wide range of parameter values (results not shown). The period of oscillations induced by PKC is related to the detachment rate of PKC.

Experiments

Experiments with PKC were performed and oscillations have been observed. First, the mixed lipid monolayer was spread on an initial pressure of 20 mN/m $\pm$ 0.5 mN/m and after the monotonous increase of the lateral pressure due to the attachment of peptide, a saturation of the monolayer is achieved, detected by an achievement of a quite constant lateral pressure (comparable to Fig. 2). The enzyme PKC was injected through the monolayer into the subphase by a syringe. The injection of PKC immediately generates a decrease in lateral pressure up to the initial pressure (results not shown). Here we assumed that the inoculation of PKC has disturbed the interface by causing a convection

of the fluid phase. Despite this disturbance, the lateral pressure increases again and oscillations have been observed over a period of hours.

The experiment was repeated with a different experimental procedure. Hereby, the monolayer was continuously compressed over the main phase transition range and stopped at a lateral pressure of $\pi = 10$ mN/m. After the peptide saturation of the monolayer, the PKC injection occurs by an injection port into the subphase, which avoids a disturbance of the monolayer. The decrease of the lateral pressure due to PKC injection could be reproduced. Next, the lateral pressure increases again and the observed oscillations of the foregoing experiment could be readily reproduced (see Fig. 6).

We have investigated monolayers at two different initial lateral pressures. The reproducibility of the observed oscillations of the lateral pressure with different experimental initial conditions shows that this process is quite robust.

The oscillations of the lateral pressure are interpreted as oscillations in the concentration of MARCKS peptide bound to the monolayer. Hereby, the amount of 0.1 μM MARCKS peptide in the subphase acts as a source for supply of unphosphorylated peptide. The characteristic period of the oscillations is in a range of 3–4 h.

Mechanism of the oscillation

Oscillations are clear indications of nonlinear phenomena. The nonlinearities of the model are associated to the binding and unbinding dynamics of peptides and enzymes. These dynamics depend on the quantity of available PIP$_2$ in the monolayer. We assume here that the characteristic size of the disordered phase channels between the ordered domains in the monolayer depends on the quantity of PIP$_2$ (see Fig. 3). The binding of MARCKS peptide changes the
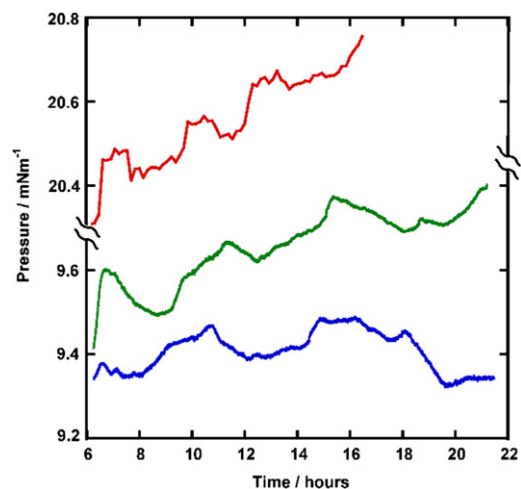

FIGURE 6 Three different experiments showing the oscillations in lateral pressure in the monolayer after introduction of PKC. (*Top*) Home-made film balance, monolayer spread-out on an initial pressure of 20 mN/m. (*Middle and bottom*) Two realizations of monolayer compressed up to 9 mN/m.

structure of the monolayer and we assume that it increases the binding rates of the peptides and the PKC enzymes.

The integration of PIP$_2$ into the DPPC monolayer yields a phase separation to an ordered condensed DPPC phase and a disordered fluid PIP$_2$-rich phase (see Fig. 3 and the sketch in Fig. 7 *a*). The interaction of MARCKS peptides with the PIP$_2$ leads to lipid/peptide clusters in the disordered phase, which causes an increase of the space between the ordered domains (23). This increase can be observed by comparison between the experimental images of the monolayers without and with attached MARCKS peptide (shown in Fig. 3, *a* and *b*). These two images correspond, respectively, to low and high values of θ in our model. The expansion of the disordered phase due to the presence of MARCKS is sketched in Fig. 7 *b*. The attachment of PKC at the interface phosphorylates the peptide (see Fig. 7 *c*) and translocates MARCKS from the monolayer into the subphase (see Fig. 7 *d*). This detachment of peptides reduces the space between the ordered lipid domains and releases PKC from the monolayer (Fig. 7 *a*); the initial situation is then recovered.

Model for open system

We have so far considered a closed system where the process of phosphorylation depletes the resources of MARCKS in the system. Living cells are open systems, which may regulate the phosphorylation of MARCKS by PKC by the following dephosphorylation of MARCKS by phosphatases (15,27). Hence, we can model an open system where the peptides can be supplied or removed and the subphase acts as a reservoir of a constant amounts of MARCKS and PKC. In such conditions, the concentration of MARCKS and PKC in the subphase is kept at constant values, M_0 and P_0, respectively. Hence, the expressions in Eq. 17 reduces to the form

$$\dot{m}(t) = k_m^+(\theta)[1 - m(t)]M_0 - k_m^- m(t) - km(t)p(t),$$
$$\dot{p}(t) = k_p^+(\theta)[1 - m(t)][1 - p(t)]P_0 - k_p^- p(t), \quad (18)$$
$$\dot{\theta}(t) = k_\theta[m(t) - \theta(t)].$$

Constant M_0 requires a continuous addition of peptides either from synthesis or external input from the outside. Alternatively, phosphatase can provide recycling of phosphorylated MARCKS peptides. This set of equations represents an open system far from thermodynamic equilibrium, which gives rise constant amplitude oscillations (Fig. 8), analogous to chemical systems maintained outside the equilibrium (28). The possibility to study such oscillations can help us to clarify the dynamics of processes involving small sets of reactants.

DISCUSSION

A better quantitative understanding of the myristoyl-electrostatic switch described in Thelen et al. (27) for living cells is the motivation of this work. The realization of well-controlled experiments is difficult in vivo. Here, we have built a reduced model system by the change of the cellular membrane, cytoplasm, and MARCKS proteins by a monolayer, a subphase, and MARCKS peptides, respectively. We keep two steps of the myristoyl-electrostatic switch—the binding of the protein to the membrane and the phosphorylation of the protein. We replace the last part of the cycle, the dephosphorylation done by phosphatases, by a reservoir of MARCKS peptide in the subphase.

Here, we have considered a simple phenomenologic model that predicts the oscillation of the lateral pressure of the monolayer. Such predictions are confirmed with experiments. We have developed a model starting from a reduced number of parameters and a particular choice of the nonlinear functions based on general arguments. Further experimental studies will be devoted to the complete characterization of the processes included in the model.
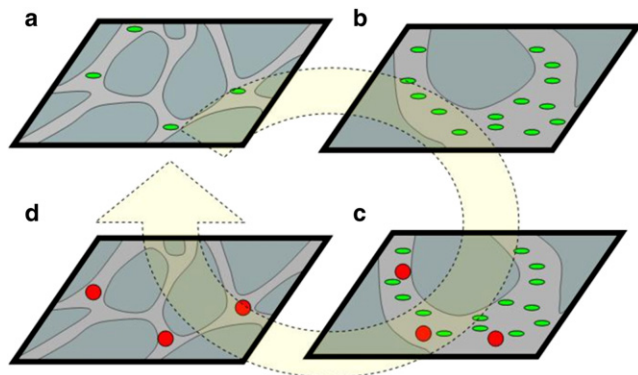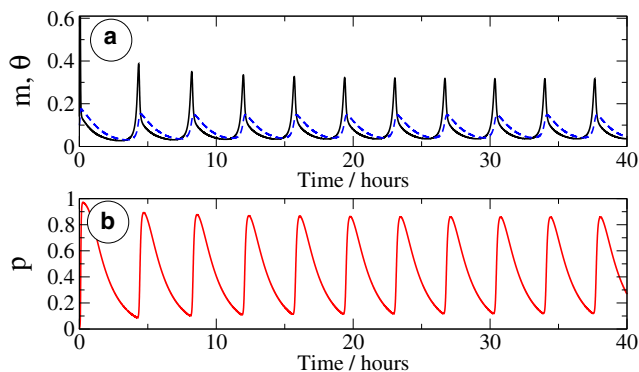

FIGURE 7 Sketch of the mechanism of lateral pressure oscillations in the monolayer. Dark-shaded areas represent the solid phase and the light-shaded channels represent the fluid phase of the monolayer. (*a*) Initial state of the monolayer, (*b*) attachment of MARCKS and increase of the size of the channels between solid domains, (*c*) attachment of PKC, and (*d*) detachment of MARCKS, reduction of the channels, and translocation of PKC.

FIGURE 8 Temporal evolution of *m* (*solid line* in *a*), *p* (*b*), and θ (*dashed line* in *a*), in the monolayer under nonequilibrium conditions during a numerical simulation. Same parameters of the numerical simulations as in Fig. 5 with $k = 0.2$ s, $M_0 = 1$, and $P_0 = 1$.

Phospholipid monolayers are reduced models of biological membranes. We have considered molecules and enzymes highly present in cells. The process of control of monolayers by the enzymes studied here is relevant for real cellular membranes, because the same ingredients are present in live cells.

The slow increase of the lateral pressure due to the attachment of MARCKS peptide at the monolayer can be reproduced by considering the diffusion of the peptide through the subphase and a simple binding dynamics to the monolayer. This process and results of experiments from the literature have been employed for the estimation of the parameters in the model.

The introduction of PKC produces a cyclic change in the monolayer. PKC induces the detachment of MARCKS from the monolayer into the subphase by phosphorylation. The posterior detachment of PKC facilitates the attachment of new MARCKS peptide at the monolayer and the repetition of the cycle. This type of interaction, combined with transport processes (diffusion), may help us to understand temporal and spatial aspects on cell signaling.

The damped pressure oscillations after the initial detachment are evidence of nonlinear interactions because kinetic oscillations are a typical signature of nonlinear processes. The model presented here shows that the feedback provided by the nonlinear binding rates of peptides and enzymes to the monolayer and the coupling of the monolayer's structure with the peptide concentration are the most important ingredients for the oscillations.

We have predicted oscillations with large temporal periods by numerical simulations and simple experiments in monolayers. The period of the oscillations mainly depends on the dynamics of the domains and fluid channels, which in our model is determined by the rate k_θ. Cellular membranes are heterogeneous and are formed by different domains. The extrapolation of our results to living cells has to take into account the possible differences of domain structure and dynamics. However, low frequency oscillations may help to regulate some of the processes controlled by the cellular membrane. Although many cellular clocks involve gene expression, the here-observed simple chemical oscillations may serve as a local clock within the cell membrane. In this article, we present an alternative mechanism for the timing of biological processes based on cyclic binding and unbinding of MARCKS proteins at biological membranes.

In summary, nonlinear interactions among peptides, enzymes, and the configuration of a monolayer produce a cyclic translocation of peptides and enzymes in the monolayer in a simple experimental setup that leads to periodic changes of the lateral pressure in the monolayer on a timescale of several hours. The oscillations are reproduced by a reaction-diffusion model by the introduction of nonlinear rates depending on the state of the monolayer. This model can be extended to conditions outside of thermodynamic equilibrium where continuous oscillations are found in simulations.

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