Interaction of dextran derivatives with lipid monolayers and the consequential modulation of the film properties

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Abstract

Polysaccharides have been associated with various biological functions through their binding to membranes, but their specific role is still under debate. The aim of this work was to study the interaction of cationic and anionic polysaccharides with anionic and zwitterionic monolayers, at different subphase compositions, thus analyzing the influence of electrostatics on the interaction. The consequent effect of the polymer-lipid binding on the film properties was studied, with special interest in monolayer dynamics. The results indicate that electrostatic interactions play an important role in polymer-membrane affinity, and that the polymers formed a sub-layer, which increased the shear viscosity of the interface. The interacting polymer, even when it did not penetrate the lipid film, induced a polymer-like behavior of the monolayer regarding its dynamics: the whole film (polymer + lipid) became very viscous. As a consequence, the dynamic of the membrane was affected, thus inducing changes in the film topography, although the energetics for phase transition and the stability of each phase were modified slightly or not at all.

Introduction:

Polysaccharides are polymeric carbohydrate molecules composed of monosaccharide units, bound together by glycoside linkages. In particular, the polysaccharide dextran comprises a family that consists of an α - (1→6) linked D-glucose main chain with varying proportions of linkages and branches. The uses of dextrans are versatile due to their solubility, biocompatibility and biodegradability[1]. The hydroxyl groups present in dextrans offer many sites for derivatization, giving rise to a large number of polysaccharides, among which are the polymers used in this study, dextran sulfate (DS, negative charged) and diethylaminoethyl-dextran (DEAE, positive charged)(see the chemical structures in figure S1). DS is a polyelectrolyte with sulfate groups along its chain, and thus the total charge density can be controlled by varying the degree of sulfation. Due to the presence of sulfate groups, DS has a wide range of applications such as anticoagulant, inhibitor of human immunodeficiency virus (HIV) and Herpes simplex virus (HSV), and as a reducer of cancer metastasis and tumor adherence[1-2], among others. The structure of this derivatized dextran is similar to the glycosaminoglycans that are present in the extracellular matrix. On the other hand, DEAE contains three basic groups with different pKa values (5.7, 9.5 and 14) [1], and thus the degree of dissociation and the conformation depends on the pH value and the ionic strength of the medium. DEAE is a biocompatible derivative with pharmacological and therapeutic properties. Its most common uses are as a reducer of bile acid and dietary cholesterol, as a non-viral vector for transfection (because it enhances the uptake of proteins and nucleic acid by the cell), in gene therapy and for drug delivery.[1, 3-4]

Polysaccharides have been associated with various biological functions by binding to the cell membrane, but their specific role is still unknown. A probably very important role of these polymers is the regulation of the membrane dynamics, as their presence affects the dynamics of lipids and proteins.[5-6]. The lateral diffusion of the components in membranes is a factor that determines, among others, the velocity of biochemical reaction-diffusion processes, and thus the function of the cell.[7] It is important to understand how these polysaccharides interact and modify membrane properties because their applications involve the interaction between polymers and the cell membrane. The influence of polysaccharides on membrane models has been studied using different approaches.[8-12] Hac-Wydro et al. show that the influence of DEAE in lipid membranes is determined by the charge of the membrane.[8] Regarding the negatively charged dextrans, Huster et al. demonstrate that DS interacts with lipids through a bridge bond with calcium, and that the magnitude of that binding depends on the chain length of the polymer and the calcium concentration[13]. Sahoo et al. and Zhang et al. demonstrate that the diffusional mobility of lipids in glucosaminoglycans-decorated membranes slowed down significantly, with the decrease being dependent on the polysaccharide concentration, the chain length and the degree of sulfation.[5-6]

The aim of this study was to investigate the modulation of the lipid film properties by its interaction with charged polymers. With this purpose, we analyzed the behavior of the film upon compression, and the interface thickness and viscosity using monolayers composed of neutral and charged lipid, thereby also studying the influence of electrostatics on polysaccharide-membrane interactions. We used monolayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-dimiristoyl phosphatidylglycerol (DMPG) in the presence of different polymers varying the ionic composition of the subphase. These lipids were selected in order to maintain the phase state while changing the film charge, especially in presence of calcium ions. Both lipids show a phase transition from liquid-expanded to liquid-condensed in the range of temperatures and of subphase compositions employed here, which makes it possible to analyze the effect of the polymer on the phase transition, as well as the features of the liquid-condensed domains. It has been proposed that the phase state is an important regulator for the interaction of soluble molecules with membranes[14-15], and therefore we prioritized maintaining the phase state and not the hydrocarbon chain length, thus choosing DPPC and DMPG for the study.

Regarding the polymers, three polysaccharides were studied: a high and a low negatively charged polymer, and a cationic polymer. The viscosity of the interface was determined in order to analyze the degree of influence of the polymer on the film's rheological properties. This was conducted by determining the Brownian motion of domains[7] or of microbeads inserted at the interface.[16] The monolayers were observed by Fluorescence Microscopy and Brewster Angle Microscopy, and thus it was possible to determine the domain shape and size in each system. We found an interesting regulation of the domain size distribution in the presence of the polymers.

Materials and Methods:

Materials:

The phospholipids 1,2-dimirystoyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (sodium salt) (**DMPG**), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (**DPPC**) and the lipophilic fluorescent probe L-α-phosphatidylethanolamine-N-(lissaminerhodamine B sulfanyl) (ammonium salt) (egg-transphosphatidylated, chicken) (Rho-egg PE) were purchased from Avanti Polar Lipids (Alabaster, Al). Dextran sulfate sodium salt of low sulfate content (3-6%, Mr~ 4.000 Da, **LDS**), dextran sulfate sodium salt of high sulfate content (from Leuconostocspp, 16-20%, Mw ~ 6.500-10.000 Da, **HDS**) and diethylaminoethyl-dextran hydrochloride (nitrogen content 2.9-3.5%, Mw~ 500.000 Da, **DEAE**) were purchased from Sigma-Aldrich.

Lipid solutions were prepared in Cl_3CH/CH_3OH 2:1 v/v to obtain a solution of 1nmol/μL total concentration, with all the solvents and chemicals used being of the highest commercial purity available. The polysaccharide solutions were prepared with deionized water (with resistivity of 18

MΩ, obtained from a Milli-Q Gradient System, Millipore, Bedford, MA) with 0.145 M NaCl (Merck Millipore - Emsure) and 0.010 M $CaCl_2$ (MERCK).

Micrometer-sized beads (0.9 μm mean diameter, carboxylate-modified latex beads, CLB9) were purchased from SIGMA.[17] The beads were cleaned by successive centrifugation and re-suspension of the pellet in clean MQ water. This procedure was repeated 10 times and then they were re-suspended in water, forming a concentrated clean bead solution (~10^{10} beads/mL). A small volume (lower than 5% of the final volume to avoid solvent phase separation) of this concentrated bead solution was added to the lipid solution, and the resulting mixture was spread at the air-water interface. The final bead density was normally about 1 bead in 2000-2500 μm^2.[16]

Methods:

Surface pressure measurements: Compression and adsorption isotherms were performed with a commercial Langmuir balance (NIMA technology), using the Wilhelmy method with a platinum plate. The compression isotherms were performed by compressing the lipid monolayers at 1 x10^{-2} nm^2 $molecule^{-1}min^{-1}$, and the monolayer was formed by spreading the lipids on the desired aqueous solution (with NaCl and with or without $CaCl_2$ and polymer) prepared at the water pH (about 6 due to the CO_2 dissolution).The temperature was adjusted by air-conditioning the room and by controlling the temperature of the trough by means of a thermocirculator (ARCTIC AC200-A10 Thermo Fisher Scientific Inc., USA). In the adsorption experiments, small drops of the lipid solution was added onto the subphase up to the desired surface pressure (in the range of 5 - 30 mN/m), and afterwards the polymer was injected in the subphase. The surface pressure value reached after 20 min of was determined.

Domain and microbead Brownian motion: Domains were tracked from videos registered using Fluorescent Microscopy (FM). For these experiments, the fluorescent probe was incorporated into the lipid solution before spreading (1 mol%). A Langmuir film balance (microtrough, Kibron) was placed on the stage of an inverted fluorescence microscope (Axiovert 200, Carl Zeiss) with a 20× objective and the monolayers were formed as explained before. Images were registered by a CCD video camera (IxonEM+ model DU-897, Andor Technology).For the analysis of the microbead motion, phase contrast microscopy was employed with the same setup as for the FM experiments. Movies of 12.16 frames.s-1and with a total length of 150 frames were taken both for bead and for domain motion. These experiments were performed at 24 ºC.

The calculation of diffusion coefficients of micrometer-sized beads (D_{beads}) and domains (D_{dom}), and the calculation of η_m from the domain motion was performed as previously detailed[7-18] (see Supplementary Material for the details).

Brewster angle microscopy (BAM): Monolayers were spread over a Langmuir film balance (minitrough, KSV Instrument) in the same manner as explained before, and they were observed

during compression using BAM with an EP^3 Imaging Ellipsometer (Accurion, Goettingen) equipped with a 20X objective (Nikon, NA 0.35). The equipment was calibrated with the clean interface (before spreading the lipid) for each experiment to be able to show the relation between the average gray-level of the images and the reflected light intensity (R_p). Reproducible images were obtained, i.e. the density, the size and the shape of the domains for different spread monolayers were similar, as well as the gray-level of the domains and the continuous phase. The analysis and quantification of domain sizes were carried out using ImageJ software as detailed in Caruso et al.[19]. The average gray-levels were calculated from the gray-levels in 6 different regions corresponding to each phase in at least 4 images for each condition.

Results and discussion:

Surface pressure - area measurements:

With the aim of understanding the effect of polysaccharides on lipid monolayers and the role played by electrostatic interactions, compression isotherms of monolayers formed by DMPG and DPPC were performed in the presence and absence of the polymers and $CaCl_2$. Three types of dextran were studied, LDS (3-6%low sulfate content), HDS (16-20% high sulfate content) and DEAE (diethylaminoethylated, nitrogen content 2.9-3.5%). Both LDS and HDS are negatively charged at the working pH (pH ~ 6), while DEAE is positively charged. The chemical structures of the polymers are shown in Fig. S1 (supporting information).[1, 20-21]The values reported for the acid constants of DEAE arepKa_1=5.7, pKa_2=9.5 and pKa_3=14.

In Figs.1a-b, representative compression isotherms of DMPG monolayers are depicted. When the DMPG monolayers were formed on solutions with NaCl and without Ca^{2+} and polymer, the surface pressure-area isotherm showed a liquid expanded/liquid condensed (LE/LC) phase transition at about 43 mN.m^{-1} at room temperature (24°C), as shown in the inset of Fig. 1a (black line). This was close to the film collapse, and it was thus difficult to differentiate both processes. Therefore, isotherms were repeated at a lower temperature. At 13°C, the phase transition was observed at 26 mN.m^{-1}, as shown in Fig. 1a (black line). When LDS or HDS were added to the subphase, no appreciable changes in the isotherms were observed at either 24 or 13ºC. In contrast, the presence of DEAE shifted the isotherms to higher mean molecular areas and, at 13°C, the phase transition was ill-defined. A similar shift to higher areas has been previously reported for DPPA and DPPG monolayers in the presence of chitosan (an aminated polysaccharide).[22-23]

In the presence of calcium ions, the DMPG monolayers showed the LE/LC transition at 18 mNm^{-1} and 24ºC (see Fig. 1b), i.e. the bivalent cation decreased the surface pressure of the phase transition, stabilizing the LC in comparison to the LE phase. This effect was previously reported by Garidel et al.,[24] who suggested that calcium ions promote a dehydration of the PG polar head-group

as a consequence of the interaction of the ion with the PG moiety, and that the neutralization of the negative charges of PG led to a maximization of the interactions between the hydrocarbon tails.

The compression isotherm of DMPG on subphases with Ca^{2+} was not affected by the presence of LDS (Fig. 1b). When HDS or DEAE were in the subphase, the transition pressure shifted to higher values (20 $mN.m^{-1}$and 25 $mN.m^{-1}$for HDS and DEAE, respectively). In the presence of DEAE, the film area shifted to higher values.

With the aim of elucidating the role of the lipid charge in the interaction of lipid monolayers with these polysaccharides, we determined compression isotherms of monolayers composed of DPPC on the same subphase compositions as those of DMPG at 24ºC (Fig. 1 a-b and Fig. S2 a-b).These monolayers showed a slight decrease in the surface pressure corresponding to the LE/LC phase transition in the presence of HDS, compared to DPPC in the absence of the polymer when Ca^{2+} was present. This effect was previously reported by Santos et al.[25] and was attributed to Coulombic interactions between DS, Ca^{2+} and DPPC. On the other hand, an increase in the collapse pressure was observed when the polysaccharides were present, possible related with over-compressed states due to a decrease in the rate of the desorption kinetic of the film in presence of the polysaccharide. Aside from those effects, no other important influence of the polymers on the DPPC monolayers was found, in agreement with previous results using DMPC monolayers [13].

The results showed so far indicate that the mean molecular area changed only for DMPG monolayers in the presence of DEAE. Therefore, we determined Gibbs adsorption isotherms by adding DEAE to the subphase once the DMPG monolayers were formed and set at a defined surface pressure, both in the absence and in the presence of Ca^{2+}. Once the polymer was injected into the subphase, the surface pressure slightly increased (the maximum increase observed was of 3 mN/m). The final concentration of the polymer in the subphase was in the range of 0.03% w/v - 0.15% w/v and the increase in surface pressure reached a plateau value (saturation) for a concentration of DEAE of 0.03% w/v (see Fig. S3a). The cut-off values for 0.10% w/v were 22 and 37 $mN.m^{-1}$in the absence and in the presence of calcium ions, respectively (Fig.S3b), which indicates that the polymer modifies the film density up to these surface pressures at each condition. The very slight increase of the surface pressure together with the slight expansion produced in the compression isotherms indicates that the polymer did not completely penetrate the DMPG film, but interacted with the lipids probably forming a sublayer, thereby affecting the mean molecular area at constant surface pressure or the surface pressure at constant mean molecular area. The formation of such a sublayer was previously reported for chitosan (another cationic polysaccharide) under anionic monolayers composed of DPPG and DMPA.[12, 22-23]

Brewster angle Images:

If a sub-layer of polymer was formed, the optical properties of the interface would change, and this can be detected with Brewster Angle Microscopy (BAM). Therefore, films of DMPG in the

absence and in the presence of $Ca^{2+}$and of the different polymers were observed by BAM. As already mentioned, DMPG monolayers displayed a phase transition upon compression which appeared at 18 $mN.m^{-1}$ at 24ºC when Ca^{2+} was present in the subphase and shifted to higher surface pressure values (close to collapse) in the absence of Ca^{2+}. Therefore, when the ion was not present, the selected experimental temperature was 13ºC, at which the phase transition was observed at 26 mN m^{-1}. As a control, the effect of the temperature change on the BAM images of the liquid expanded phase of DMPG on 0.145 M NaCl was measured and the results are shown in Fig. S4.

The presence of the first order phase transition was detected with BAM by the emergence of domains of different shapes. Fig. 2 shows representative BAM micrographs for DMPG monolayers on the different subphase compositions and at different surface pressures. In panel a, no Ca^{2+} was present in the subphase and the temperature was 13ºC, whilst in panel b, the subphase contained 0.010 M $CaCl_2$ and the temperature was 24ºC.

At both ionic composition and temperature, the presence of LDS did not modify the film features. When HDS was present, no major changes were observed; this polymer in the presence of calcium ions slightly increased the surface pressure for domain emergence, as also observed in the compression isotherms (fig. 1b). In the presence of DEAE, the gray level of the images markedly changed, both in the presence and in the absence of Ca^{2+}. At 13ºC and without $CaCl_2$, domains were not detected even at high surface pressures, whilst at 24ºC and 0.010 M $CaCl_2$, domains were small and rounded and appeared at higher surface pressures in agreement with the compression isotherms (Fig. 1b).

Fig. 3 shows reflected light intensity (R_p) as a function of lateral pressure for images of DMPG monolayers, such as those presented in Fig.2. The value of R_p depends on the interface refractive index and on its thickness,[26] and therefore, a change in R_p reflects a change in these parameters. We cannot quantify the film thickness since the refractive index is unknown, and therefore we compared the R_p values as a qualitative measure of the thickness, and an indication of the presence or absence of concentrated polymer at the interface. In all the experimental conditions at which domains were observed, an abrupt change in the R_p values was detected: the LC phase always showed higher values of R_p than the LE phase as a consequence of its greater thickness and lower refractive index.[26] Furthermore, during compression, the R_p value for the LC phase remained roughly constant, corresponding to slight changes in the molecular density and tilting upon compressing the lipid at this phase, whilst an appreciable increase was detected in the LE phase upon compression as a consequence of higher changes in the molecular density and order during compression.

A comparison of the black symbols in Fig. 3 a and b indicates that the R_p values decreased when Ca^{2+} was present in the subphase. In the absence of this ion, the R_p values for the LE and LC phases were in the ranges of 2-5 $\times$ 10^{-7} and of 1 $\times$ 10^{-6}, respectively, while when the ion was

present, R_p shifted to 10^{-8} - 10^{-7} for the LE phase and to $8\times10^{-7}$for the LC phase. This change was not caused by the change in temperature, as an increase from 13 to 24ºC on NaCl solutions promotes negligible changes (see Fig. S4). As we mentioned, the interaction of calcium ions with the PG moiety was previously reported as inducing a partial dehydration of the lipid polar head-group, thus resulting in a decrease in the interfacial thickness.[27]

Fig. 3a shows that, in the absence of calcium, LDS and HDS did not affect the film reflectivity, in agreement with the absence of influence on the compression isotherms (Fig. 1 a and b) and on the average domain shapes and sizes (Fig. 2a). In contrast, the presence of DEAE markedly changed the reflectivity of the interface, inducing an increase in the R_p value of one order of magnitude at all lateral pressures, and the abrupt change in R_p in the region corresponding to the phase transition was absent, matching the absence of domains (see Fig. 2a).

The increase in the interface reflectivity in the presence of DEAE, together with the slight shift to higher areas in the compression isotherms (Fig. 1 a and b) and the slight increase in surface pressure in the penetration experiments (Fig. S3a), clearly indicate that, although the polymer did not noticeably penetrate the monolayer, it interacted with the lipids, generating a thicker interface. Thus, a layer of polymer was formed below the DMPG monolayer, affecting the observation of the domains. Similar results were found for the interaction of other polysaccharides with amino groups.[22]

In the presence of calcium, the R_p value was not affected by LDS, while HDS and DEAE increased its value. HDS increased the LC more markedly than the LE optical thickness, suggesting that it interacts differently with a denser and more organized lipid phase. It has to be recalled, however, that the R_p values in the presence of $CaCl_2$ and HDS were similar to the values determined in the absence of both molecules. These results may be interpreted then as a loss of calcium from the PG moiety due to competition with the sulfate groups of the HDS. In the absence of these ions, the PG polar head group would rehydrate, thus recovering the optical thickness observed when this ion was absent. In this context, Huster et al.[13] demonstrated that the affinity between dextran sulfate and Ca^{2+} is of the same order as that of the phosphate groups of DMPC and Ca^{2+}.

However, the phase transition occurred at lower surface pressures in the presence of Ca^{2+} and the polymer than in their absence, although it shifted to slightly higher values when the polymer was added, compared to subphases with Ca^{2+} and without HDS. In other words, the presence of this polymer only partially reversed the effect of calcium ions on the surface pressure corresponding to the phase transition, with the values changing from 43 mN/m (without Ca^{2+}) to 18 mN/m (with Ca^{2+}) and to 20mN/m (with Ca^{2+} and HDS). This seems to indicate that HDS formed a sub-layer below the DMPG monolayer in the presence of Ca^{2+}. This is in agreement with the results reported in Huster et al.[13], where the presence of a calcium bridge between PC and DS was demonstrated by ^2H Nuclear Magnetic Resonance. Also, Mejere et al. found an increase in the thickness of the

interface for the DPPE monolayer in the presence of Ca^{2+} and DS,[28] and additionally, a similar interaction has been reported between a negative polysaccharide and DHP mediated by Zn^{2+}.[29]

The cationic polymer DEAE also increased film reflectivity in the presence of calcium, despite reaching lower $R_p$values than in its absence, indicating that the polymer-lipid layers were thinner and/or that the refractive indexes changed in the presence of the bivalent ion, and therefore, that the sub-layer properties were modulated by Ca^{2+}.

Domain and microbead Brownian motion:

The presence of a polymer sublayer may very possibly affect the rheological properties of the interface. In order to test this hypothesis, we analyzed the motion of species inserted in the DMPG monolayers in the presence and absence of calcium ions and of the different polymers. The Brownian motion of species at the interface depends on the subphase and on the monolayer viscosity in an indirect fashion, and this dependence was previously used both to determine the monolayer viscosity[7, 16, 30-31] and to compare the rheological properties of different films.[15]

The use of domain motion is preferred since the calculation of the film viscosity from their diffusion coefficients is more straightforward than from those of beads,[16] but if the same temperature at all conditions is explored, no domains are present in the absence of Ca^{2+}at 24ºC and a foreign probe must be added. Therefore, in this condition, the Brownian motion of latex microbeads was analyzed and the diffusion coefficients of the beads were compared in the absence and in the presence of the different polymers.

Fig. 4 shows the histograms of the values for the diffusion coefficients of domains (a) and beads (b) at all the analyzed subphase compositions and at 24ºC for the DMPG monolayer, and the average values are shown in Table I. In all cases, a purely viscous behavior was observed, with a linear variation of the mean square displacement with the time lapse (see Fig. S5). In Fig. 4a, the red line on the histograms indicates the limit for a diffusion controlled by subphase viscosity (see Supplementary Material, section 3), whilst in Fig 4b the lines indicate the range of values for D_{bead} previously reported for the DMPC monolayer on NaCl solutions, i.e. D_{bead} values for similar beads inserted in an LE monolayer on a non-specifically interacting subphase.[16] From Fig. 4 and Table I, we conclude that the diffusion coefficients shifted to lower values in the presence of HDS and of DEAE, following the order: **without polymer ≈ LDS>HDS>DEAE**.

These shifts may be caused not only by an increase of the interface viscosity, but also by an increase in the subphase viscosity, since the motion of micrometer sized beads and domains depends on both viscosities. Therefore, we determined the diffusion of beads on a clean interface (in the absence of the DMPG monolayer) using the different subphase compositions. The average diffusion coefficient for beads, both in the absence and in the presence of polymers, was $(4 \pm 2).10^{-13}$ $m^2.s^{-1}$, without appreciable influence of the subphase composition. These values were similar to

those found in Wilke et al.[16] on clean NaCl subphases. Additionally, the diffusion of domains was analyzed in DPPC monolayers, and the values obtained were insensitive to the presence of the polysaccharides, with all of them in the range of (1 – 4).10^{-13} m^2s^{-1} (see Fig S6 and S7). Therefore, the presence of the polysaccharides *per se* (in the absence of DMPG) did not affect the diffusion of the domains and of the microbeads.

Furthermore, to additionally check the possible changes in subphase viscosity at all compositions and at both temperatures used in the BAM experiments and in the compression isotherms, the bulk viscosity of the solutions was determined (see Section 7 of the Supplementary Material). Values of 0.7-0.8 and 1 N s m^{-2} were obtained at 24 and 13ºC, respectively, for all solutions (see Fig S8 and Table S1).

The monolayer viscosities η_m for DMPG in the presence of the different polysaccharides and Ca^{2+} (estimated from the domain diffusion coefficient as explained in the Supplementary Material)are shown in Table I. As expected, this parameter increased in the order: **without polymer ≈ LDS <HDS<DEAE**. The values found for the viscosity are in the range of those found for fluid monolayers at low-intermediate surface pressures using micro-particle tracking-techniques,[7,16,30-34] and lower to those found with commercial rheometers[35-36]. This kind of discrepancy between the techniques was already pointed out in the 60´ [37] and it is still unresolved[38].

Domain size and film viscosity:

A notable result shown in Fig. 2 was that domains showed different shapes and sizes in some conditions. Fig. 5a summarizes the domain features during phase transition; in general, large domains were flower-like, while small domains were rounded. Since the selected images correspond to surface pressures with a similar amount of area of each phase (about 50%), larger domains imply a lower domain density, as shown in Fig. 5b.

Domain shape transitions have been widely reported[39-45], as well as the related driving forces. A flower-like domain shape opposes the requirements of line tension and may be caused by two very different mechanisms: by non-equilibrium domain growth, called diffusion-limited aggregation (DLA),[39] or by an equilibrium shape transition, due to electrostatic repulsions within the molecules inside the domains, which was fully described by McConnell (see[40] and references therein). In both cases, domains are expected to be rounded when they are small and elongated as they grow larger, as observed here. The different domain shapes shown in Fig. 5a are thus a consequence of the differences in the number of domains that led to different sizes at comparable percentages of phase transition. Therefore, we now focus on the reasons for the differences in the number of domains.

In the experiments shown here, domains appeared and then grew as the film was compressed, without appreciable changes in the number of domains during compression. In other words, we did

not observe the merging of domains, new domains budding from a large one or the generation of new domains after the nucleation stage, which means that the number of domains was defined during the nucleation stage. During nucleation, unstable clusters of the LC phase are generated by density fluctuations, and grow to become stable clusters once their radii are larger than a critical radius.[46,47] If a large number of clusters reach the critical radius at the same time, the domain density will be high, and thus, domain density depends on the speed of the perturbation rate in relation to the film dynamics.[42, 47-49] Here, we used the same perturbation rate (film compression) in all experiments, and the thermodynamic properties for the phase transition (enthalpy and entropy change) did not appreciably change when the subphase composition was varied (see Table S2).The most remarkable change promoted by the polymers was found in the rheological properties, and thus, even though the compression rate was similar, the film dynamics changed in the presence of DEAE or HDS. If lipid mobility is hindered, migration of the molecules to the closer nucleation point is precluded, and thus new nuclei will generate at a point close to the initial positions of the molecules forming it.

The viscosity of the interface varied in the order: DEAE<HDS<LDS≅without polymer at 24ºC, both in the presence and absence of Ca^{2+} (see Fig. 4 and Table I). We assume that this tendency is conserved at lower temperatures. Fig. 5b shows that the domain size varied in the order: HDS≥LDS≅without polymer for domains in the absence of Ca^{2+} and at 13ºC. In the presence of DEAE, domains were not observed, probably because their sizes were below resolution (1 μm). In order to test this, we compressed DMPG monolayers in DEAE solutions at the slowest available compression rate (0.24 x 10^{-2} $nm^2.molec^{-1}.min^{-1}$) and, although they were very small, domains were observed during this experiment (see inset in Fig. 5a), and they appear at the same surface pressure than in the DMPG film in absence of DEAE. We therefore conclude that the lipid motion was the determining step for nucleation of the liquid-condensed phase in DMPG monolayers in the absence of $Ca^{2+}$and at 13ºC.

In contrast, interface dynamics was not a determining step for nucleation of the liquid-condensed phase of DMPG monolayers in the presence of Ca^{2+}at 24ºC, with and without HDS or LDS, since changes in the viscosity of the interface did not affect the monolayer texture. However, for subphases with DEAE and Ca^{2+}, the film viscosity was high enough to influence nucleation, translating to a greater number of smaller domains (Fig 5b).

Conclusion:

In this work, we studied the role played by electrostatics in the polymer-lipid interaction, and the modulation of film properties induced by this interaction. An influence of electrostatic interactions in the monolayer-polymer affinity can be concluded from the following results: *i.* All polymers affected more markedly the properties of DMPG than of DPPC films; *ii.* The system with polymer and monolayer with opposing charges showed the highest effects (DEAE-DMPG); and *iii.*

The anionic polymers influenced the DMPG films in the presence of Ca^{2+} and only in the case of the highly charged polymer (HDS). It should be remembered however that, according to Huster et al.[13], the DS-PC interaction depends, among other factors, on the polymer length (amount of monomers) and, since the HDS used has an average length greater than that of LDS, the differences found between HDS and LDS may be due not only to the density charge difference but also to the different polymer sizes. In the presence of calcium, HDS and DMPG showed an interaction seen in the change in transition surface pressure, reflectivity and film viscosity. However, the mean molecular area of the isotherms remained the same when the polymer was added. This lack of change may be due to the low molecular weight of the dextran derivative used (6.5-10 KDa) since, as already stated, the effect of DS is highly dependent on the average molecular weight of the polysaccharide.[13]

Regarding the consequences of the polymer-lipid interactions in the film properties, the BAM experiments indicate that a sub-layer of the polysaccharides was generated (DMPG with DEAE with and without Ca^{2+} and DMPG with HDS in the presence of Ca^{2+}). This sub-layer appears not to markedly affect the film compression isotherm (Fig. 1); only slight changes were observed in the mean molecular areas (in the presence of DEAE with and without Ca^{2+}) and/or the surface pressure corresponding to the phase transition (in the presence of DEAE or HDS and of Ca^{2+}). This translates to negligible effects on the thermodynamics of the phase transition (see Table S1). In contrast, the polymer layer below the lipid monolayer markedly increased the interface viscosity, affecting the film dynamics. This translated to an effect on domain nucleation during the first stages of phase transition, and thereby, to different film textures (different domain sizes and shapes). Additionally, a blurring of the phase transition was observed in the compression isotherms of DMPG in DEAE solutions (Fig. 1b), as previously reported for fast compression in relation to film dynamics.[50-51]

In summary, when polymers interacted with the lipid monolayers, a sub-layer was formed, affecting principally the film viscosity. In cell membranes, polysaccharides interact via proteoglycans with the outer leaflet of the cytoplasmatic membrane. In this context, our results may relate with a possible role of polysaccharides, as regulators of the membrane dynamics without strongly affecting the global energetics. It has been demonstrated through Monte Carlo[52] and Molecular Dynamics simulations[53] that some immobilized lipids in a sea of molecules, such as the membrane, act as static obstacles for the remaining mobile lipids, accounting for the small domains described in the plasma membrane. Therefore, the kinetics of the processes occurring in the membrane may be modulated, among other manners, through these polymer-membrane interactions.

Regarding the use of polymers for controlled drug delivery, the results found here indicate that a foreign polymer could affect the cell activity when interacting with its membrane, despite not noticeably affecting the energetics of other processes. Here we showed in a model system, which is simple and in equilibrium, how a modification in the film viscosity may affect the monolayer texture.

In a complex and out-of-equilibrium system, such as a living cell, effects on membrane dynamics are very probably translated to much more important changes in the whole system.

Supplementary Material

1- *Structure of the polymers*
2- *Surface pressure - average molecular area compression isotherms of DPPC*
3- *Domain and microbead Brownian motion:* Details for the experimental determination of particle motion and film viscosity.
4- *Gibbs adsorption isotherms of the polymers into monolayers of DMPG.*
5- *Reflectivity of the interface with DMPG at different temperatures.*
6- *Supplementary information for the bead and domain diffusion data.*
7- *Bulk viscosities of the subphases.*
8- *Enthalpy and entropy changes for the phase transitions.*

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