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Two-dimensional Physical Networks of Lipopolymers at the Air/Water Interface

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SUMMARY: The objective of this manuscript is to review recent film balance and interfacial rheology experiments on Langmuir monolayers of lipopolymers and lipopolymer/phospholipid mixtures at the air-water interface. In film balance experiments, we have observed that the high-film-pressure transition occurring at about 20 mN/m for mixtures containing between 40 and 100% lipopolymer, which is related to a first-order-like alkyl chain condensation, is a necessary requirement for the existence of a rheological transition. At this rheological transition, which is observed with a novel interfacial stress rheometer, dramatic increases in both the storage and loss moduli occur as the area per lipopolymer molecule is decreased. We have shown that these transitions are observed for lipopolymers prepared from both poly(ethylene glycol) and poly(ethyl oxazoline) polymer backbones. The combination of film balance and surface rheology experiments is interpreted in terms of the formation of a quasi-two-dimensional physical network in which there are two essential intermolecular interactions to impart elasticity. The first involves condensation of terminal hydrophobic alkyl chains into small aggregates at the air side of the Langmuir film. The second involves hydrogen bonding between segments of the hydrophilic polymer coil in the water subphase, mediated via water bridging.

Introduction

In the context of our experiments, we define a lipopolymer as a macromolecule having a backbone composed of hydrophilic repeating units and hydrophobic long-chain, aliphatic moieties attached either randomly in multiple locations along the chain or at a single chain end. For appropriate combinations of hydrophilic backbone and hydrophobic side chains or terminus, the macromolecule will be surface-active and will form a stable monolayer at the air-water interface. The mechanical response of such a monolayer to compression/expansion cycles in a classical Langmuir film balance can yield considerable information about the various intramolecular and intermolecular interactions among the macromolecule and the water subphase. Moreover, under suitable conditions, the monolayer may be transferred to a

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solid substrate by the so-called Langmuir-Blodgett-Kuhn technique and used to create a supramolecular assembly.

Typical hydrophilic polymer backbones for lipopolymers include poly(ethylene oxide) or poly(ethyl oxazoline), which may be partially hydrolyzed to form the copolymer poly(ethyl oxazoline - co - ethylene imine). The aliphatic hydrophobic moiety is generally at least 16 carbons in length and is most effective in imparting surface activity when attached to the backbone as a dimer. One synthetic approach leading to such a molecule is to use dioctadecyl amine as a chain initiator in a ring-opening polymerization of ethyl oxazoline. Another approach is to react a dialkyl-glycero-phosphoethanolamine with the carboxy terminus of a poly(ethylene oxide) chain. Single alkyl chains may also lead to amphiphilic character. Thus, a single chain amine could be used as an initiator or terminator of a reaction.

Lipopolymers have recently been at the focus of several classes of studies with biophysical or biomaterial orientation. For example, lipopolymers are being used as model systems to study the properties of end-grafted polymers [1-3]. For some time, there has been intensive theoretical and experimental interest in such systems, with the focus largely on the chain configuration of the surface-attached polymer as it extends out into a solvent, which may be thermodynamically good or poor. The visually descriptive nomenclature commonly used to describe these macromolecules includes the "pancake", "mushroom", and "brush" structures, with the configurations depending upon the grafting density and upon the solvent quality. Experimental approaches to prepare such grafted systems have relied extensively on diblock copolymers, with one of the blocks being collapsed in a dense layer on the solid substrate, acting as an anchor for the other block projecting out into solution. In fact, the lipopolymer at the air-water interface is another example of a system that can be used to study chain configuration because the lipid moiety anchors the hydrophilic polymer chain to the air-water interface. Thus, the surface density may be easily controlled by lateral compression of the film.

When a chloroform solution of a lipopolymer, e.g. one based on poly(ethylene glycol) (PEG), is first spread on a water surface and the spreading solvent is allowed to evaporate, the lipopolymer may be considered to form a complex, disordered film lying entirely in the plane of the air-water interface. Lateral movement of the confining barriers and measurement of the resulting surface tension leads to a surface pressure-area isotherm. Such isotherms typically exhibit two transition plateaus for PEG lipopolymers -- a low-film-pressure transition at about 10 mN/m, which is related to the submerging of PEG chains from the air-water interface into the subphase [1], and a high-film-pressure transition. As a result of IR experiments on selectively deuterated lipopolymers, Wiesenthal et al. showed that the high-film-pressure transition is related to a first-order-like alkyl chain condensation [4]. These same authors had observed earlier that only lipopolymers carrying a hydrophobic anchor of saturated alkyl chains exhibit a high-film-pressure transition, whereas those with unsaturated chains show no such transition [2]. The latter behavior was also found for polystyrene-PEG diblock copolymers [5].

In a second example, lipopolymers have been used in the molecular engineering of complex, polymer-tethered phospholipid bilayers [6,7]. In such a process, a mixture of a lipopolymer and a single phospholipid is spread at the air-water interface, compressed to an appropriate surface pressure, and then transferred to a solid substrate when the substrate is drawn through the monolayer. A feedback system permits maintenance of the surface pressure and, hence, the same molecular packing during the transfer process. If the substrate, which is typically a silicon wafer with a native oxide surface or a glass microscope slide, has been previously functionalized with a photoactive silane coupling agent, it may be possible to covalently bond the polymer to the solid substrate through irradiation of the assembly following transfer. We have reported on an effective coupling agent of this type based on benzophenone [8]. Following the irradiation, we envision that the assembly consists of a monolayer of alkyl chains (with unknown defect content) attached to the solid substrate via a water-swollen, gel-like polymer cushion. The final step of the supramolecular assembly involves the deposition of a top lipid leaflet via horizontal Schaefer transfer of a Langmuir film of a lipid or lipid mixture or via vesicle fusion. Current efforts focus on the evaluation of the lateral mobility of the free lipids in both the lower and upper leaflets, which may be important for maintenance of biological function of integral membrane proteins that may be embedded in the bilayer in various projected biodiagnostic applications.

The objective of this paper is to review recent experiments in surface viscoelasticity of lipopolymers and their mixtures with phospholipids at the air-water interface, as developed in a series of three papers by Naumann et al. [3, 9, 10]. Such dynamic measurements complement the spectroscopic and reflectivity measurements that have much more commonly been used to interrogate Langmuir films. This work was made possible in our laboratories with the recent development of a novel interfacial stress rheometer , which permits determination of the interfacial storage and loss moduli [11]. Other techniques for measuring the rheological properties of amphiphiles at the air-water interface include deep-canal devices, channel-flow devices, and rotating disks or rings [12, 13]. In the following, we will describe the basic characteristics of our interfacial stress rheometer, present typical dynamic results in comparison with film balance measurements, outline the variations in molecular structure that have been examined, and present a general interpretation of the dynamic results in terms of the development of a two-dimensional physical network.

Approach

Interfacial Stress Rheometer

Major components of the interfacial stress rheometer consist of a pair of Helmholtz coils surrounding a mini-Langmuir trough (KSV Instruments) [11], as shown in Figure 1.

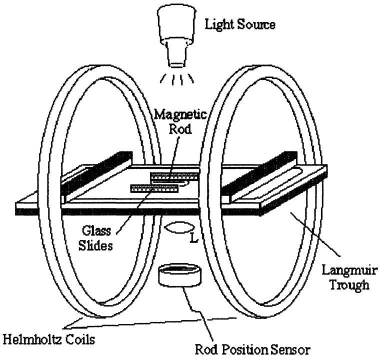

Fig. 1: Schematic of the interfacial stress rheometer (ISR) used to measure the mechanical shear properties of Langmuir monolayers. With this device, a magnetized rod is subjected to an oscillatory force generated by a pair of Helmholtz coils, and the resulting motion is detected using a microscope and photodiode array. From measurements of the amplitude ratio and phase of the applied force to the resulting displacement, the dynamic surface moduli ($G_s^* = G_s' + iG_s''$) can be detected. The storage modulus, G_S', is a measure of the elasticity of the monolayer, and the loss modulus, G_S'', is a measure of the viscous nature of the film. {Figure adapted from Figure 2 of reference 3.}

A small, magnetized rod is supported at the air-water interface by surface tension, aligned along the axis of the Helmholtz coils by two parallel glass walls, and subjected to an oscillatory force generated by the coils. The needle responds to the oscillatory stress with an oscillatory strain detected by an optical microscope and photodiode array. The needle moves at the same frequency as the imposed oscillatory force, but differs in phase, from which information about the viscoelastic character of the interface may be obtained. In particular, we determined the delay between the stress and the strain and the ratio of their amplitudes. These two measurements allowed determination of both the storage modulus, G_S', which represents the elastic properties of the monolayer, and the loss modulus, G_s'', which represents the viscous behavior. The viscoelastic properties were independent of strain (0.005 to 0.07) for all films studied, indicating linear viscoelasticity. Frequency-dependent experiments were occasionally carried out over the range from 0.5 to 10 rad/sec. More commonly, however, we performed viscoelastic measurements as a function of film pressure (equivalent to a particular surface area per molecule) with the frequency maintained constant at 0.92 rad/sec. In different experiments [3, 9, 10], temperatures have been varied over a modest range from 10 to 30°C, pH has been varied from 2.0 to 5.5, and a D_2O subphase has been examined. We will only consider room temperature, pH 5.5, water subphase experiments in this paper.

Lipopolymers

We have examined a variety of lipopolymers in our experiments, including 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol) 2000] (DSPE-EO45), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol) 5000] (DSPE-EO110), dioctadecyl amine [poly(ethyloxazoline) 4032] (DODA-E35), dioctadecanoylglycerol-[poly-2-ethyl-2-oxazoline 3710] ($DC_{18}Gly-E_{31}$), and dioctadecanoylglycerol-[poly-2-methyl-2-oxazoline 3640] ($DC_{18}Gly-M_{35}$). Thus, we have been able to compare directly the effect of the length of the hydrophilic polymer chain (DSPE-EO45 vs. DSPE-EO110) and the effect of the chemical structure of the hydrophilic polymer chain ($DC_{18}Gly-E_{31}$ vs. $DC_{18}Gly-M_{35}$). We have also been able to compare, somewhat indirectly, the relative amphiphilic character of a dialkyl amine terminal hydrophobic group and that of a phospholipid terminal hydrophobic group, but we do not have the precise lipopolymer pair for the ideal comparison. In some experiments, we have examined mixtures of lipopolymers with phospholipids. Typically, we have used 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) because this phospholipid does not show a phase transition at the air-water interface. A lipid phase transition would overlap with that of the lipopolymers, thus making a careful analysis of the lipopolymer transition behavior more complicated.

Representative Results

Pure Lipopolymer Monolayer Films

The key results, which are representative for all of our experiments on different lipopolymers, are shown in Figure 2. In this case, the lipopolymer under study is 1,2-distereoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol)2000] (DSPE-EO45). This figure compares

Wilhelmy plate film balance measurements (dashed-dotted line) with viscoelastic measurements of the storage modulus G_s' (filled circles) and loss modulus G_s'' (open circles).

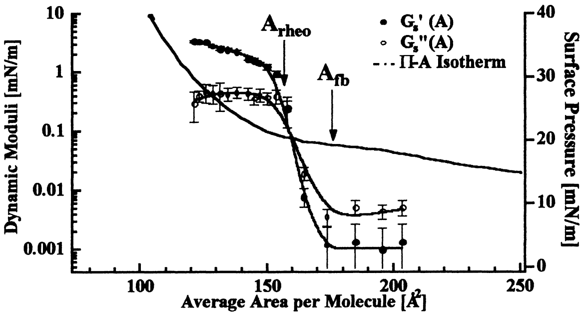

Fig. 2: Correlation of the increase in storage modulus G_s' and loss modulus G_s'' with the high-pressure transition, π_{high}, observed in π-A isotherms. The inflection point in the isotherm in the high-pressure region is identified as the film-balance transition (A_{fb}), and the cross-over point (where $G_s' = G_s''$) is called the rheological transition (labeled A_{rheo}). $\pi(A_{rheo}) - \pi(A_{fb})$ is typically 1 mN/m. When the area per molecule is less than A_{rheo}, G_s'' plateaus, whereas G_s' increases with increasing surface density.

The results for both sets of measurements are plotted in terms of area per molecule. Several observations can be made about these viscoelastic data:

1. At a particular value of the area per molecule, A_{rheo}, both the storage and loss moduli undergo large transitions; the storage modulus G_s' increases by over three orders of magnitude as the area is reduced and the loss modulus G_s'' increases by about two orders of magnitude. This viscoelastic transition region is relatively narrow, with a width of approximately 15 Å^2/molecule.

2. For average molecular areas less than A_{rheo}, the complex modulus is dominated by its elastic contribution, i.e. $G_s' > G_s''$, whereas for average molecular areas greater than A_{rheo}, the complex modulus is dominated by its viscous contribution, i.e. $G_s'' > G_s'$.

3. A correlation appears to exist between A_{rheo} and the inflection point of the pressure-area isotherm at the high pressure plateau, A_{fb}. We recall that Wiesenthal et al. have attributed the high-film-pressure transition to an alkyl chain condensation [4], so the pure lipopolymer experiments might suggest that the rheological and film balance transitions have the same molecular basis. However, since the two transitions do not occur at precisely the same point,

the question of the molecular basis for the high-film-pressure and rheological transitions cannot be unequivocally determined from the pure lipopolymer results.

The rheological transition is, however, not a PEG-specific phenomenon, as additional surface rheology experiments on poly(alkyl oxazoline) lipopolymers ($DC_{18}Gly\text{-}M_{35}$ and $DC_{18}Gly\text{-}E_{31}$) have shown [9]. Figure 3 illustrates that $DSPE\text{-}EO_{45}$, $DC_{18}Gly\text{-}M_{35}$, and $DC_{18}Gly\text{-}E_{31}$ show almost the same surface rheological behavior if G_s' and G_s" are considered on a reduced area scale, $A_r = (A_{rheo} - A)/A_{rheo}$. All three lipopolymers compared here not only have the same alkyl chain length, but also polymer chain lengths of 45, 35, and 31 monomer units, which are too similar to explain the observed differences. Our data indicate that the observed rheological transition is a general property of monolayers of lipopolymers even if polymer moieties form a different polymer-water complex; however, the absolute value of A_{rheo} differs for different lipopolymers.

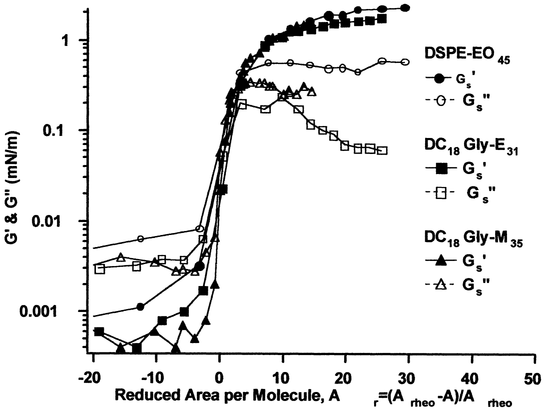

Figure 3: Surface rheological behavior of $DSPE\text{-}EO_{45}$, $DC_{18}Gly\text{-}E_{31}$, and $DC_{18}Gly\text{-}M_{35}$ plotted on a reduced area scale, $A_r = (A_{rheo} - A)/A_{rheo}$. Although the viscous behavior is slightly different among the observed lipopolymers for $A < A_{rheo}$,, the corresponding elastic response is the same.

Phospholipid/Lipopolymer Mixed Monolayer Films

While our initial measurements provided clear evidence of a dramatic change in the rheological properties in the vicinity of the high-film-pressure transition, we were interested in examining the apparent correlation between A_{rheo} and A_{fb} in more detail. To do this, we prepared mixtures of DMPC and DSPE-EO45 with DMPC compositions ranging from 0 to 70 mole percent [10]. All films were examined with fluorescence microscopy (labeled with 1% NBD-labeled phospholipids) and there was no indication of any macroscopic phase separation. Thus, the phospholipids act as neutral spacer molecules, thereby changing the packing and stress conditions between adjacent PEG chains. The results of these experiments are presented in Figure 4, where pressure-area isotherms of several DMPC/DSPE-EO45 mixtures (solid lines) are shown along with values of A_{fb} (squares) and A_{rheo} (circles).

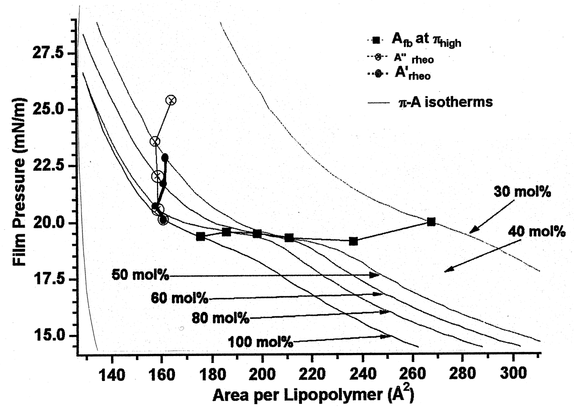

Fig. 4: Comparison between high-film-pressure and rheological transitions of DMPC / DSPE-PEG2000 mixed monolayers at the air-water interface at lipopolymer molar concentrations of 30 / 40 / 50 / 60 / 80 / 100 mol%. The π-A isotherms (solid lines) show the points, where the rheological transition can be observed (loss modulus: A''_{rheo}, storage modulus: A'_{rheo}), and the inflection points of the transition plateau at the high-film- pressure transition (A_{fb} at π_{high}). There is a clear correlation between the occurrence of the rheological transition and the existence of the high-film-pressure transition. {Figure taken from Figure 7 of reference 10.}

Significant observations from these results are as follows:

1. The admixture of the DMPC phospholipid into the DSPE-EO45 lipopolymer Langmuir film does not change the shapes of the pressure-area isotherms; rather they appear to be simply shifted laterally to larger areas per lipopolymer. The inflection point of the pressure-area curve in the region of the high-film-pressure transition remains constant at approximately 20 mN/m, but A_{fb} shifts to larger values as the phospholipid content increases.
2. In spite of the large change in the pressure-area isotherms for the mixtures, the rheological transition occurs at a fixed A_{rheo} of approximately 165 Å^2 , independent of the amount of incorporated phospholipid; this is true for both the storage and loss moduli. The fact that the rheological transition does not occur in the same region as the high-film-pressure transition indicates that the two transitions must have different molecular origins.
3. No rheological transition is observed for mixtures with greater than 70 mole percent phospholipid content. Under these same conditions, the high-film-pressure transition no

longer is apparent in the pressure-area isotherm. Thus, it appears that the high-film-pressure transition is a necessary precursor for the existence of the rheological transition.

Interpretation

The dramatic increase in storage and loss moduli at A_{rheo} coupled with the change in character from a viscous to elastic dynamic response are consistent with the formation of a physical gel network [3, 9, 10]. The critical question, however, is the nature of the molecular mechanism underlying the network formation. It is well-known that the hydrophilic character of poly(ethylene glycol) results from a specific structuring of water molecules along the polymer chain and the formation of hydrogen bonds between ether oxygens and the surrounding water molecules [14, 15]. Thus, it seems quite reasonable to assume that similar intermolecular interactions might be occurring in the DSPE-EO45 and DSPE-EO110. Moreover, since similar rheological behavior [9] is observed for the oxazoline-based materials, $DC_{18}Gly-E_{31}$ and $DC_{18}Gly-M_{35}$, we are led to the conclusion that the basic molecular mechanisms must also be similar. Guenet and coworkers first proposed the possibility of the formation of a physical gel by polymer-solvent complexes [16, 17]. Although Guenet focused on different polymer-solvent systems, we have adopted his reasoning to interpret our results [3, 9, 10]. For simplicity, we will discuss this in terms of Langmuir films of the pure lipopolymer.

Our argument proceeds as follows: At effectively zero surface pressure, the lipopolymer chains lie in the plane of the air-water interface, and there are no intermolecular interactions between either the hydrophobic or the hydrophilic polymer repeat units. As the Langmuir film is compressed, a low-film-pressure transition occurs in the vicinity of 10 mN/m, which is accepted as being associated with the desorption of the polymer from the air-water interface into the water subphase [1]. In response to further compression, the mushroom-like polymer coils in the water subphase are expected to become stretched to avoid interpenetration, although there does not appear to be any feature in the pressure-area isotherms that can be specifically attributed to a mushroom-brush transition. The critical aspects of our proposal revolve around the molecular interactions that occur at high compression when the polymer chains are stretched (thus reducing the projected area per chain) and the terminal lipid groups are closer together.

We first consider hydrophobe-hydrophobe interactions and recall that the high-film-pressure transition has been attributed to an alkyl chain condensation [4] and that this transition is necessary [9, 10] in order for a rheological transition to occur.

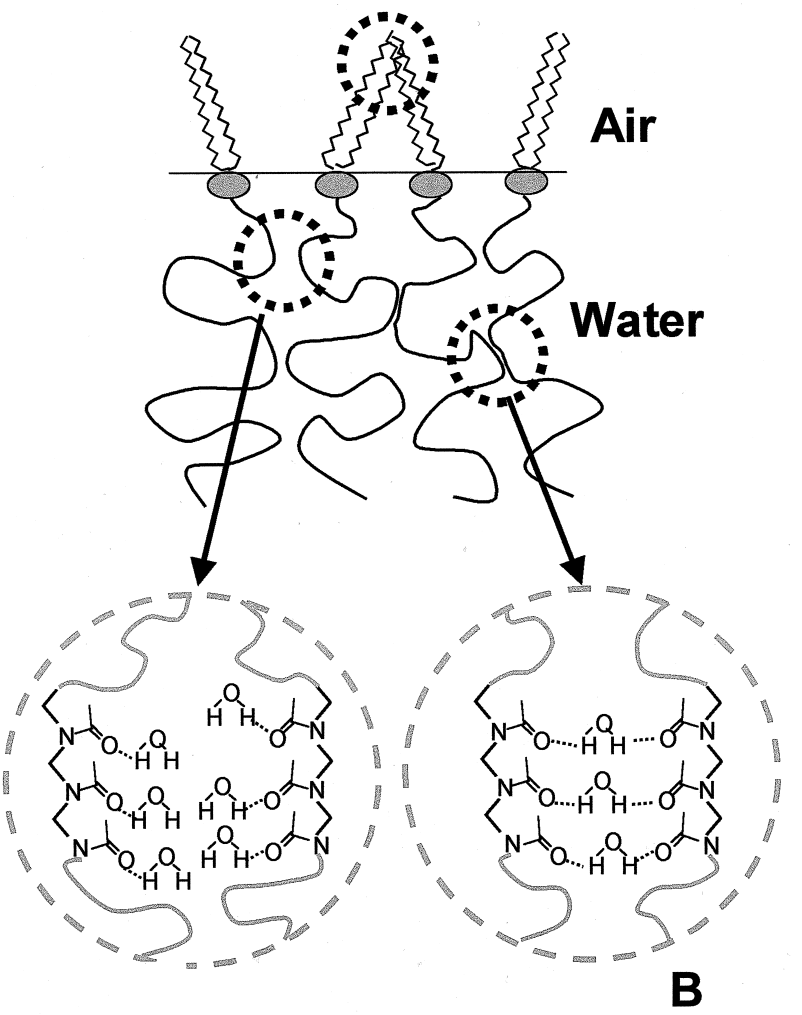

Fig.: Schematic of a possible molecular model to explain the observed physical network formation among poly(methyl oxazoline) lipopolymers. As the lipopolymer molecules are compressed to a brush-like configuration beyond A< A_{rheo}, two regions should be distinguished: (A) the chain is hydrated with water molecules at locations where there are vacant H-bonding sites exposed to bulk water, or (B) the hydrated water molecules act as intercalates, linking two neighboring chains via hydrogen bonding. These physical junction points are not permanent, leading to the observed reversible character of the rheological transition. The model for the physical gelation of poly(ethyl oxazoline) lipopolymers is assumed to be similar (not shown). {Figure taken from Figure 5 of reference 9.}

The extent of alkyl chain association must be limited in the lipopolymer monolayers, however, because of the significantly larger cross-sectional area of the polymer (125 – 170 Å^2 for DSPE-EO45) compared to the terminal lipid moiety (about 62 Å^2). A reasonable estimate may be that a microdomain of associated terminal lipid groups would consist of alkyl chains from two to four lipopolymers [M]. This is illustrated in Figure 5. Although the occasional formation of clusters of extended lipopolymer brushes temporarily bound by the alkyl chain condensates is well-justified by the experiments of Wiesenthal et al. [4], it is insufficient to explain the rheological transition without an additional type of intermolecular interaction. Even if all terminal alkyl chains were located in microclusters, steric restrictions would lead simply to a collection of small clusters of associated lipopolymers, not an infinite network. Some alternate molecular mechanism that involves the hydrophilic polymer coils in the water subphase must be invoked. We find this through consideration of hydrogen bonding.

Throughout these initial stages of compression, we believe that the hydrophilic polymer coils are fully hydrated with water molecules. At still higher degrees of compression, however, we propose that water molecules are “squeezed out” of the polymer-water complex, leading to water bridges between segments of different polymer chains. This aspect of our proposal is also shown in Figure 5, where inset A is meant to reflect fully hydrated segments of the polymer coil and inset B is meant to reflect the water bridges formed upon expulsion of some of the intervening water molecules. We have no detailed picture of the coil configuration in the vicinity of these proposed water bridges. Nevertheless, the similarity of the rheological transition behavior for poly(ethylene glycol) and polyethyloxazoline based lipopolymers suggests that the intermolecular interactions may be similar. Assuming this to be true, we then propose that water bridging between segments of a polymer coil belonging to a particular microcluster and those of a coil in a different microcluster provides the additional contact necessary to form the network. Note that intramolecular bridging between segments on the same coil or intermolecular bridging between coils in the same microcluster will not be effective in network formation and will not lead to any monolayer elasticity.

Outlook

The monolayer behavior of lipopolymers and their mixtures with phospholipids provides a rich field for additional structural and dynamic studies. Of particular interest is the continued examination of our proposal that water bridging between highly extended polymer chains is an important element leading to the formation of the elastic physical network. Future conferences on polymer-solvent interactions may shed light on this intriguing phenomenon.

Acknowledgments

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