Part 2. Crossover from Depletion Attraction to Adsorption: Polyethylene Glycol Induced Electrostatic Repulsion between Lipid Bilayers

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ABSTRACT: Using the surface forces apparatus, we have measured the static and hydrodynamic forces between two substrate-supported lipid bilayers in water and semidilute, aqueous solutions of poly(ethylene glycol) (PEG), a neutral polymer for which water is a good solvent. In part 1 (previous paper in this issue), we presented detailed measurements of depletion attraction in the presence of 8000 molecular weight PEG. In part 2 (this work), we address the behavior of higher molecular weight 20 000 PEG. Specifically, the higher molecular weight PEG adsorbs to the surfaces and both a steric as well as an unexpected electrostatic repulsion were measured. We determined that higher MW PEG chelates metal cations and thereby induces an apparent electrostatic charge on the bilayer surfaces upon adsorption.

Introduction

The adsorption of poly(ethylene glycol) (PEG) at interfaces is an important phenomenon in numerous practical applications, since many aqueous colloidal systems are stabilized or destabilized by nonionic polymer additives of which PEG is one of the most common. In part 1 of this work,[1] we described how the addition of certain molecular weights of PEG led to an enhanced attractive force between surfaces through a depletion attraction effect. In this second paper, we focus on the crossover from depletion to adsorption as a function of increasing molecular weight of the polymer.

That longer chains adsorb to a surface in comparison to the depletion of shorter, but otherwise identical, chains is not particularly surprising. Very simply, polymer adsorption is governed by the adsorption energy, ϵ, per monomer of the chain. When the total energy exceeds kT, $\epsilon_{\text{total}} \approx n\epsilon > kT$, where n is the total number of adsorbed or bound monomers, polymer chains will adsorb to the surface. In effect, there is a "critical" chain length above which a sufficient number of segments form physical bonds with the surface.[2] Moreover, since the entropy per chain in the bulk is given by $S \propto \Phi_b/N \ln(\Phi_b/N)$, where Φ_b is the monomer bulk volume fraction and N is the number of monomers per chain, large chains lose less entropy than smaller chains upon adsorbing to a surface and are thus preferentially adsorbed.[3]

The structure and interaction of such adsorbed polymer layers have been studied extensively both experimentally and through theoretical treatments.[4] The crossover from depletion to adsorption although straightforward from a theoretical viewpoint is a difficult region to probe experimentally. Here, we present direct and quantitative studies of this crossover in aqueous solutions of PEG. The "critical" chain length or molecular weight occurred over a surprisingly narrow range, 8000−20 000. In addition, the behavior in a mixed solution of 8k and 20k PEG clearly demonstrates both phenomena, depletion of the lower 8k MW PEG chains and adsorption of the higher 20k MW PEG chains. This combination of interaction forces has previously been referred to as "hairy" depletion attraction.[5]

Experimental Section

Chemicals and Sample Preparation. Dimyristoyl phosphatidylcholine (DMPC) and dipalmitoyl phosphatidylethanolamine (DPPE) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). PEG-8000 (8k) was from Sigma Chemical Co. (St. Louis, MO), PEG-18,500 (18k) and PEG-20 000 (20k) were from Polysciences, Inc. (Warrington, PA), and PEG-20 000 (20k-h) was from Shearwater Polymers, Inc. (Huntsville, AL). All chemicals were used without further purification. PEG is water soluble at moderate temperatures in all proportions over a wide range of molecular weights (MWs).

Traditionally, commercial grade 8k PEG is used to aggregate and fuse biological colloids. Although lower MWs can also be effective at very high concentrations, higher MWs, 18k or higher, are found to be ineffective. Thus, the behavior is very MW-dependent in this range. The 8k (Sigma) and 18k and 20k (Polysciences) PEG we use are commercial grade and polydisperse. Although the 8k PEG is a homogeneous chain of (CH_2-CH_2-O) terminated by hydroxyl groups, the 18k PEG is produced by reaction of two ~9000 MW chains with bisphenol A diglycidyl ether.[6] Hence, the higher molecular weight chains are slightly chemically different from lower molecular weight samples (Figure 1). To account for this difference we also used commercial grade 20 000 MW PEG from Polysciences that is chemically identical to the lower MW 8000 PEG. Finally, monodisperse homogeneous PEG 20k-h (Shearwater) was also investigated. This polymer does not contain the reactive center of the traditional high MW products, but differs from the Polysciences homogeneous PEG in that it is very monodisperse, <1.06.

Static Force Measurements. The Surface Forces Apparatus, Mark II, was used to conduct all force measurements. This apparatus has been described previously.[1,7]

Model bilayer surfaces were constructed on the mica substrates used in the Surface Forces Apparatus by Langmuir−Blodgett deposition. The first or inner layer of the supported bilayer was DPPE ($\Pi = 40$ mN/m, 43 Å^2/molecule), which binds physically but strongly to the mica substrate. DMPC ($\Pi = 31$ mN/m, 58 Å^2/molecule) was deposited as the second or outer

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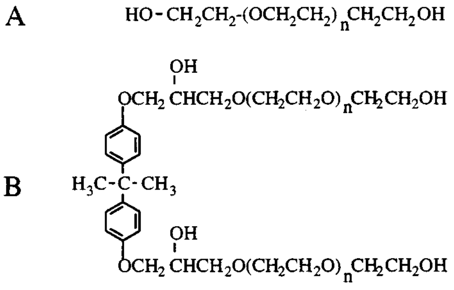

Figure 1. (A) Chemical structure of low molecular weight poly(ethylene glycol) (PEG) also commonly referred to as poly-(ethylene glycol). (B) Chemical structure of 18k PEG constructed by linking two lower molecular weight ~9000 MW PEG chains via reaction with bisphenol A diglycidyl ether. High MW PEG usually contains such linker groups.

layer. DMPC was chosen because it is commonly used in PEG-induced aggregation and fusion experiments of vesicles.[8] After the DMPC layer was deposited, the disks were transferred under water to the measuring apparatus and mounted, as described elsewhere.[9] Prior to the insertion of the bilayer-coated disks, the apparatus was filled with 0.5 mM KNO_3 water or PEG solution saturated with free DMPC to prevent lipid desorption from the bilayers during the experiments. Throughout this work, the surface separation, $D = 0$, was defined as contact between nominally dehydrated bilayers (cf. Figure 1, part 1[1]).

Viscosity Measurements. The same apparatus as used in static force measurements was used to measure the dynamic interaction of the two bilayers immersed in water or PEG solutions. Specifically, the dynamic technique enables measurement of the viscosity, μ, of the intervening fluid as a function of surface separation. Details of this technique are given in part 1.[1]

Results and Discussion

Static Force Measurements. General Overview. Figure 2 gives a general overview of the normal force profiles measured between lipid bilayers in water as well as in aqueous solutions of 8k and 18k MW PEG at 10 wt %.[10] All measurements were made at 25 °C. In the absence of polymer, the neutral lipid bilayers weakly adhere to each other via a van der Waals interaction with an energy minimum at $D \approx 30$ Å, below which repulsive hydration and steric protrusion forces oppose closer approach.[9] In the presence of 8k MW PEG at 10 wt %, the measured adhesive minimum occurs at the same separation ($\approx$30 Å) but is significantly greater than the always present van der Waals attraction. The increased adhesion is due to the depletion interaction in the presence of nonadsorbing "free" polymer chains.[1,10] However, there is also an unexpected repulsive force at larger separations ($D \approx 300$ Å or $4R_F$) prior to the attractive regime. Conversely, with PEG 18k at 10 wt % the interaction is entirely repulsive at all separations due to confinement or adsorption of this particular molecular weight to the bilayer surface. As a result, the surfaces remain separated by a 67 ± 2 Å thick layer of compressed polymer even under a compressive force of 50 mN/m.

Adsorption of Higher MW PEG. The entanglement molecular weight for PEG is 17 600. Hence at a concentration of 10 wt % of PEG 18k ($C^* = 1.6\%$), molecular entanglements with an appreciable lifetime may exist and individual molecules no longer diffuse separately. As a result, the PEG 18k chains may remain kinetically trapped in the gap between the approaching surfaces. The measured repulsive force between the surfaces would then be due to the entropic confinement and elastic compression of the trapped chains. To determine whether the 18k chains were truly adsorbed to the bilayer surface or merely kinetically trapped between the approaching surfaces, we also investigated a much lower concentration of 1 mg/mL, or 0.1 wt % PEG 18k. As seen in Figure 3A, even in the dilute regime, a strong repulsion is measured between the surfaces and the position of hard contact is shifted out by 15 ± 5 Å indicating that PEG 18k adsorbs to the bilayer surface. Hence, chains of this particular molecular weight *adsorb* to the bilayer surface. Moreover, others have shown that vesicle tumbling rates, measured with ^{31}P NMR, and vesicle aggregation are both inhibited in the presence of PEG 18k,[10,11] again indicating that this is an equilibrium effect.

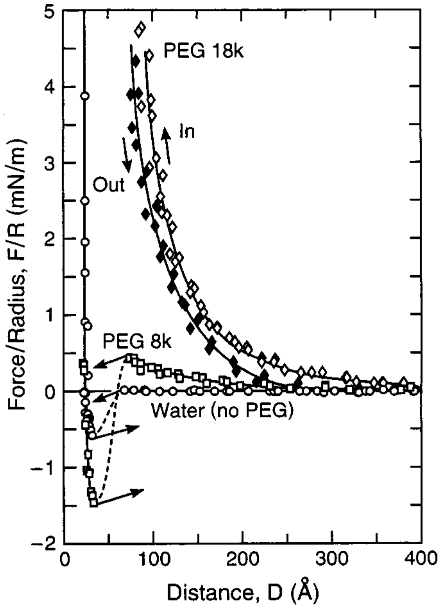

Figure 2. Force profiles, F/R vs D, between supported DPPE/DMPC bilayers in water and aqueous PEG solutions. The circles and dashed curve are the force profile in water, i.e., in the absence of polymer, where the bilayers adhere due to van der Waals attraction. The bilayers are prevented from approaching closer than $D \approx 20$ Å by the strong short-range steric–hydration repulsion. The arrows indicate when the spring constant is exceeded by the gradient of the attractive force. The resulting mechanical instability causes the surfaces to jump together or apart. Thus, these portions of the force profile are inaccessible. Squares: force profile in PEG 8k at 10 wt %. The adhesion between the surfaces is significantly greater in the presence of the polymer due to an attraction depletion interaction. Diamonds: open symbols are for the approach in PEG 18k while filled symbols are taken during the separation. Note that the force with the higher molecular weight polymer is long range and entirely repulsive.

However, as shown in Figure 1, PEG 18k is chemically different from lower molecular weight chains, such as PEG 8k, since 18k PEG is produced by reacting two lower MW (~9000) chains via a reactive linker of bisphenol A diglycidyl ether (multiple linkages are used to make higher MWs).[6] Thus, higher MW chains generally have a hydrophobic center group. Although such groups are not expected to bind to a hydrated bilayer surface, to elucidate this issue, we also investigated the interaction with homogeneous PEG 20k chains (mondisperse 20k-h and polydisperse 20k), which are chemically identical to PEG 8k. Once again, a strong repulsion was measured between the surfaces

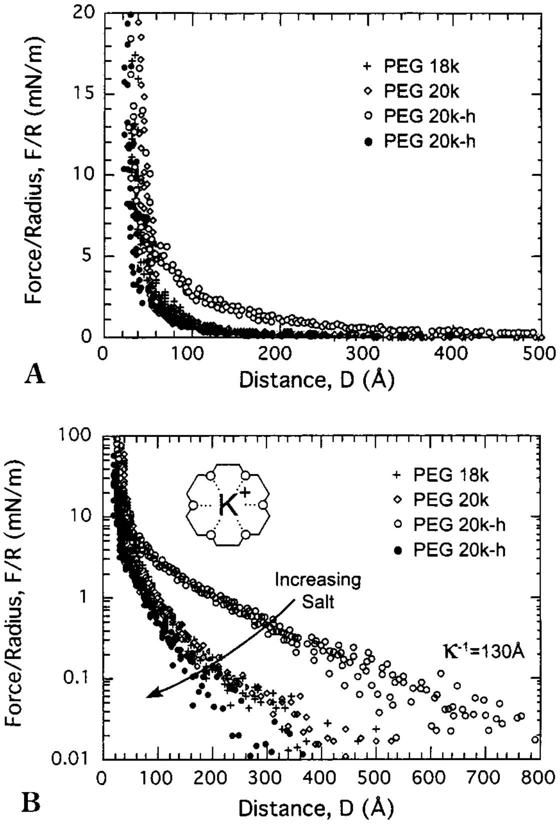

Figure 3. Force profiles measured between bilayers in the presence of 0.1 wt % PEG with a nominal molecular weight of 20k. In all cases, the higher molecular weight polymer adsorbs to the surfaces and the position of contact is shifted out by 25 ± 10 Å as compared to in the absence of polymer. (A) Linear plot: Plus symbols are the data for PEG 18k from Polysciences in 5 mM KNO_3. This polymer contains a hydrophobic linker as shown in Figure 1. Diamonds are for polydisperse PEG 20k from Polysciences, which does not have the hydrophobic linker group. Open (0.5 mM KNO_3) and filled circles (5 mM KNO_3) are the data measured with monodisperse PEG 20k from Shearwater. This polymer is homogeneous and does not contain a hydrophobic linker group. (B) Same data as shown in part A but on a semilog scale. The long-range repulsion measured with the higher molecular weight PEG is due to an electrostatic interaction. The apparent surface charge is 25 ± 5 mC/m^2. Inset: schematic of a six member crown ether ring binding K^+. It is more likely that a less structured $(-OCH_2\text{-}CH_2-)_n$ loop is formed around the metal cation. The "perfect" six member ring is shown for illustrative purposes.[12]

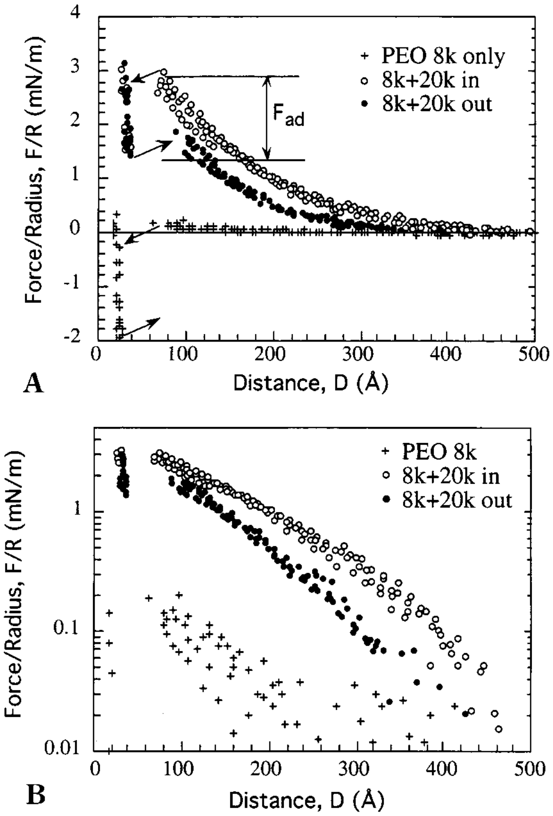

Figure 4. (A) Plus symbols are measured in PEG 8k at 10 wt %. The depletion interaction leads to an enhanced attractive minimum which occurs at the same separation as in the absence of polymer. Circles: upon the addition of 0.1 wt % PEG 20k, the force profile is dramatically altered. The position of contact shifts out by 20 Å due to the adsorption of the higher molecular weight PEG. However, a depletion interaction due to the free PEG 8k chains is still measured and the surfaces jump in (open symbols) and out (filled symbols)of contact. The adhesion is about the same as measured prior to the addition of PEG 20k. (B) Same data plotted on a semilog scale. The long range, exponential force is due to a repulsive electrostatic interaction between the surfaces.

indicative of chain adsorption even at a low concentration of 0.1 wt % (Figure 3A). These results indicate that the repulsive force measured in solutions of higher MW PEG is most likely due to single chain adsorption, and that PEG chains of these molecular weights are sufficiently long ($\epsilon_{\text{total}} \approx n\epsilon > kT$) to bind individual chains to the surface.[2] However, when these data were plotted on a semilog scale (Figure 3B), it became clear that the source of the repulsion was not only from a steric interaction, due to PEG adsorption, but also from a weak long-range electrostatic interaction.

Long-Ranged Electrostatic Repulsion. To verify the electrostatic origin of the long-range repulsion at 0.1 wt % PEG 18k, 20k, and 20k-h, the electrolyte concentration was varied during some experiments. As shown in Figure 3B, the exponential decay length decreases from 130 Å at 0.5 mM to 40 Å at 5 mM KNO_3 as expected for the Debye length. At first glance, these results are rather unexpected since PEG is a nonionic polymer. However, PEG itself is not becoming ionized. Rather, some of the chains are chelating metal cations. A six-membered ring of crown ethers happens to have the right size of binding pocket to chelate metal cations, especially sodium and potassium (inset of Figure 3B).[12] Note, all experimental measurements were conducted with a background electrolyte concentration of 0.5 or 5 mM KNO_3. Similar results were obtained if $NaNO_3$ was substituted for KNO_3. Thus, higher MW PEGs bind to the membrane surface, but they also carry chelated metal cations with them, thereby rendering the surfaces positively charged. Moreover, this apparent surface charge is relatively high, 25 ± 5 mC/m^2, indicative of the high affinity of PEG to both bind to the bilayer surface and chelate metal ions at this particular molecular weight. We are currently investigating whether these phenomena are related.[13]

"Hairy" Depletion Attraction. To further elucidate the issue of higher MW chains binding to the surface and the resulting electrostatic interaction, we also investigated the behavior of a mixed polymer solution. Initially, the experiment only contained PEG 8k at 10 wt % as shown by the crossed data points in Figure 4A. A small amount of 20k-h was added to obtain a concentration of approximately 1 mg/mL PEG 20k-h. (Note, it is not possible to know the exact concentration of the added 20k-h in the gap between the bilayer coated

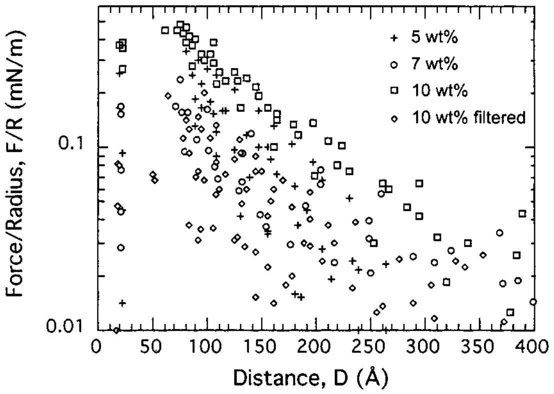

Figure 5. Semilog plot of the data measured in PEG 8k at 5, 7, or 10 wt %, including results presented in part 1 of this work. The weak repulsion appears to have an exponential decay.

surfaces, due to the difficulties of mixing the viscous polymer solution, 8k at 10 wt % $\mu = 4.3$ cP, in situ inside the SFA.) As seen in Figure 4A, there was a marked effect on the force profile upon addition of the higher MW chains. A longer range, $D \cong 450-500$ Å, and steeper repulsion are now measured. However, sluggish jumps in to and out of contact are still present. The magnitude of the adhesion is still consistent with the bulk solution osmotic pressure due to the free PEG 8k chains in solution. However, the position of bilayer–bilayer contact is pushed out by 15 Å, compared to the contact in the absence of 20k-h. Hence, some of the PEG 20k-h remains on the bilayer surfaces in contact. The hysteresis seen upon separating the surfaces from the adhesive contact is most likely from the expulsion or rearrangement of some of the PEG 20k-h chains in the gap region during the compression and subsequent jump into contact.

Finally, the total magnitude of the adhesion is about the same with an absorbed PEG 20k-h layer as measured previously only in the presence of PEG 8k at 10 wt % ($F_{ad}/R \approx 2$ mN/m). This is consistent with the additivity of the repulsion force due to confinement of absorbed chains with the attractive depletion interaction, which is frequently referred to as "hairy depletion".[5] The presence of an absorbed PEG 20k-h layer effectively pushes out the position of contact, but the depletion interaction still occurs between the now polymer-coated surfaces and the nonabsorbing free PEG 8k chains in solution. The long-range electrostatic interaction becomes very obvious when the same data as shown in Figure 4A is plotted on a semilog scale (Figure 4B). Again, the 20k-h layer adsorbs to the surface, carrying some chelated metal cations.

Repulsion in PEG 8k Revisited. As mentioned previously, a very weak repulsion was measured with PEG 8k before the attractive depletion interaction. The data, including part 1 of this work, is collected on a semilog plot in Figure 5. When displayed in this format, the repulsive barrier in PEG 8k also appears to have an electrostatic origin in some of the measurements (square data points). Because of the polydispersity in the commercial grade PEG samples, the question arises as to whether the repulsion in the case of PEG 8k is due to aggregates or to a few stray higher molecular weight chains. Although, PEG 8k is quite polydisperse, $M_w/M_n \cong 1.9$, the maximum MW range is fairly shallow.[12] Using GPC, we did not detect any MW chains above 23k in PEG 8k supplied by Sigma. Conversely, Shearwater's PEG 20k-h is monodisperse, $M_w/M_n \cong 1.06$. It is remarkable that there is such a dramatic difference in adsorption behavior over this a narrow molecular weight range, 8k–20k. Even though MWs as high as 85k were detected in GPC runs of the PEG 20k-h, it seems unlikely that the large adsorption we measured would be due solely to these stray, higher MW chains. As shown in parts A and B of Figure 4, a small amount of PEG 20k-h, 1 mg/mL, altered the force profile tremendously from that when just PEG 8k was present. Depletion attraction is the dominant interaction observed in PEG 8k, while 20k PEG adsorbs on the bilayer surface.

In summary, the interaction of the polymer with the surface is dictated by a combination of many factors, including the polymer molecular weight, concentration, presence of contaminates, and the background electrolyte.

Dynamic Viscosity Measurements. In colloidal systems with high concentrations of free polymer in solution *and* an adsorbed layer of polymer at the surface, "hairy" depletion attraction has been observed where the colloids flocculate due to an attractive osmotic difference and exclusion of free polymer from the region between the colloids (ref 5 and Figure 4). At the most simplified level, the adsorbed polymer is modeled by merely shifting the hard wall of the colloidal particle out by the thickness of the adsorbed layer. Hence, depletion of the free chains still occurs as the separation of the colloids decreases and a resulting flocculation of the colloid particles occurs even in the presence of an adsorbed polymer layer. This interaction was directly measured in the mixed system of PEG 8k at 10 wt % when 0.1 wt % of PEG 20k-h was added (Figure 4).

Conversely, the static force measurements with 10 wt % PEG 20k were entirely repulsive, and no attraction or adhesion was measured between the surfaces (Figure 2). In other words, although a depletion interaction from the nonadsorbed or free PEG 20k chains may have been anticipated, such an interaction was not detected in the static force measurements. In contrast, our dynamic viscosity measurements (Figures 6 and 7) show a significant reduction of the fluid viscosity in the whole gap for separations less than 1200 Å as the depletion layer dominates the flow, similar to that seen in the PEG 8k measurements (part 1,[1] Figures 4 and 5). At smaller separations, below 200 Å, Figure 7 shows that the viscosity of the gap solution rapidly increases as fluid flow through the adsorbed polymer layers now dominates. According to Figure 6, at large separations ($D > 200$ Å) the *effective* shear plane is negative ($D_{shear} = -60$ Å), indicative of the presence of a depletion layer.[1] However, for smaller separations $D_{shear} = +80$ Å, indicative of a shift in the shear plane due to the adsorbed polymer layers.

This viscosity profile is consistent with the phenomena of "hairy" depletion.[5] However, we did not directly detect any long-range depletion attraction between the surfaces in the presence of PEG 20k. This is not to say that no attraction due to depletion occurs in the system; possibly the repulsion due to the compression of the adsorbed polymer layer, electrostatic interaction, or presence of adsorbed polymer aggregates overwhelm the attractive interaction. Theoretically, to first order, the steric repulsion due to an adsorbed layer of polymer is expected to decay with a power law of 2, $F/R \propto D^{-2}$.[14] The power law decay in PEG 20k at 10 wt % was 3.0.

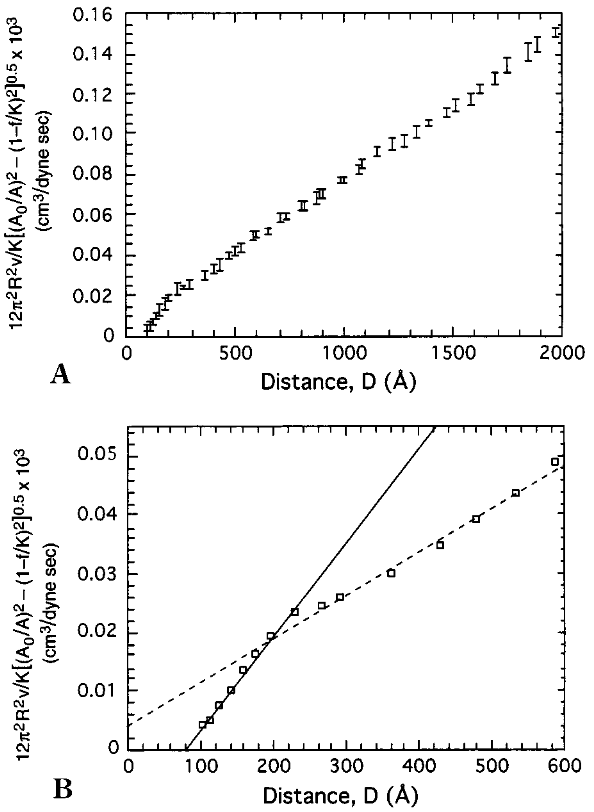

Figure 6. (A) Plot of $(12\pi^2R^2\nu)/K[(A_0/A)^2 - (1 - f/K)^2]^{1/2}$ against distance D in 10 wt % PEG 18k at 25 °C. Ideally, if the viscosity equals the bulk value at all distances, the points should fall on a straight line whose inverse slope gives the viscosity. The intercept of the line with the D axis occurs at the plane of shear. The error bars are the standard deviation for each measurement. (B) Same as part A but in more detail; curves are linear regressions to the data. Dashed curve: the shear plane at large separations is negative, $D = -60$ Å, possibly due to a "hairy" depletion interaction. Solid curve: at smaller separations the shear plane shifts out to $D = +80$ Å, indicative of an absorbed polymer layer on the surface.

The apparently more rapid decay in the repulsion may be due to the hidden attractive depletion interaction. However, this is purely speculative, and we have not been able to resolve the absence of attraction in the static force measurements with the dramatic reduction in viscosity at intermediate separations shown in Figure 7.[14] We leave the investigation of this surprising viscosity effect for future work.

Conclusions

PEG 20k adsorbs on the bilayer surface, resulting in a short-range steric repulsion and a long-range electrostatic interaction. At first glance, these results are rather unexpected since PEG is a nonionic polymer. However, the chelation of metal cations by PEG, although not well-known in the colloidal science community, has been well established in the field of organic chemistry.[12] We are currently investigating whether the adsorption to the bilayer surface was driven by the chelation and the apparent positive charge of the PEG chain or whether a noncharged chain could adsorb independently. It seems likely that association with a surface would stabilize the chelation of a charged ion. Last, our dynamic viscosity measurements indicated that at intermediate surface separations ($D > R_F$) the gap solution viscosity in PEG 20k was significantly reduced compared to that of the bulk, a characteristic of "hairy" depletion attraction. At smaller separations, the viscosity increased as expected due to the adsorbed polymer layer.

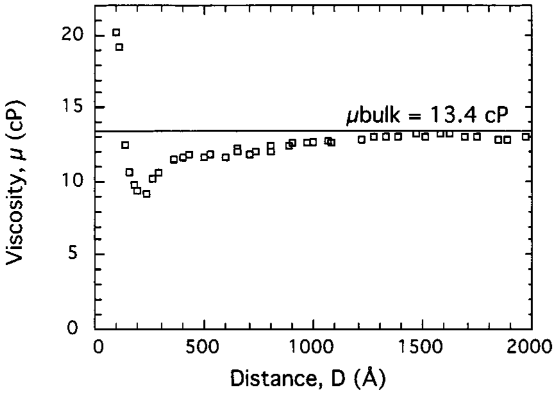

Figure 7. Viscosity as a function of distance calculated using eq 1, part 1. For separations greater than 1200 Å, the viscosity is as expected for a bulk sample of PEG 18k at 10 wt %. Below 1200 Å, the viscosity falls, possibly due to a "hairy" depletion interaction. At small separations, the viscosity rapidly increases as the absorbed polymer layers on the surfaces interact and greatly impede the flow of fluid from the gap.

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References and Notes

(1) Preceding paper: Kuhl, T. L.; Berman, A. D.; Hui, S. W.; Israelachvili, J. N. *Macromolecules* **1998**, *31*, 8250–8257.

(2) Klein, J.; Pincus, P. *Macromolecules* **1982**, *15*, 1129–1135. Equations III.9–III.13 define the critical condition at which polymer adsorbs to a surface. The critical condition is determined by the parameter β which is proportional to $1/R_g$ or $1/(\mathrm{MW})^{1/2}$, and on $\ln(1/\phi_b)$ where ϕ_b is the bulk polymer concentration. For the same binding energy per segment, the critical condition is therefore sensitive mainly to the segment number and less so (through the log-dependence) on the concentration. Thus, below some critical polymer concentration there is ZERO adsorption, above it there is finite (even quite high) adsorption. While this paper applies to poor solvent conditions, the general ideas are believed to also apply to theta and good solvent conditions. See also: Ploehn, H. J. *Colloids Surf. A* **1994**, *86*, 25–40.

(3) Strictly speaking, this argument is not completely correct because the loss in translational entropy is not the only driving force.

(4) Scheutjens, J. M. H. M.; Fleer, G. J. *J. Phys. Chem.* **1979**, *83*, 1619–1635. de Gennes, P. G. *Adv. Colloid Interface Sci.* **1987**, *27*, 189–209. Patel, S. S.; Tirrell, M. *Annu. Rev. Phys. Chem.* **1989**, *40*, 597–635. Takahashi, A.; Kawaguchi, M. *Adv. Polym. Sci.* **1982**, *46*, 1–65. Garvey, M. J.; Tadros, Th. F.; Vincent, B. *J. Colloid Interface Sci.* **1974**, *49*, 57–68.

(5) Vincent, B.; Luckham, P. F.; Waite, F. A. *J. Colloid Interface Sci.* **1980**, *73*, 508. Rao, I. V.; Ruckenstein, E. *J. Colloid Interface Sci.* **1985**, *108*, 389. Vincent, B.; Edwards, J.; Emmet, S.; Jones, A. *Colloids Surf.* **1986**, *18*, 261. Van Lent, B.; Scheutjens, J. M. H. M.; Fleer, G. J. *J. Colloid Interface Sci.* **1990**, *137*, 380–394. Wijmans, C. M.; Zhulina, E. B.; Fleer, G. J. *Macromolecules* **1994**, *27*, 3238–3248.

(6) Dave Jordan, Sigma Chemist. Personnel communication.

(7) Israelachvili, J. *J. Colloid Interface Sci.* **1973**, *44*, 259. Israelachvili, J.; Adams, G. *Nature (London)* **1976**, *262*, 774.

(8) Blow, A. M. J.; Botham, G. M.; Fisher, D.; Goodall, A. H.; Tilcock, C. P.; Lucy, J. A. *FEBS Lett.* **1978**, *94*, 305. Tilcock, D. P. S.; Fisher, D. *Biochim. Biophys. Acta* **1982**, *688*, 645.

Boni, L. T.; Hah, J. S.; Hui, S. W.; Mukherjee, P.; Ho, J. T.; Jung, C. Y. *Biochim. Biophys. Acta* **1984**, *775*, 409. Boni, L. T.; Hui, S. W. In *Cell Fusion*; Sowers, A., Ed., Plenum Press: New York, 1987; p 301. Arnold, K.; Hermann, A.; Pratsch, L.; Gawrisch, K. *Biochim. Biophys. Acta* **1985**, *815*, 515.

(9) Marra, J.; Israelachvili, J. *Biochemistry* **1985**, *24*, 4608.

(10) Kuhl, T. L.; Guo, Y.; Alderfer, J. L.; Berman, A. D.; Leckband, D.; Israelachvili, J. N.; Hui, S. W. **1996**, *12*, 3003−3014.

(11) Rupert, L. A. M.; Engberts, J. B. F. N.; Hoekstra, D. *Biochemistry* **1988**, *27*, 8232.

(12) Cross, J. Ed. *Nonionic Surfactants*; Marcel Dekker: New York, 1987. There is an entire chapter entitled Metal ion complexes of polyoxyalkylene chains in Bailey, F. E., Jr.; Koleske, J. V. *Poly(Ethylene Glycol)*; Academic Press: New York, 1976. We have independently determined that PEG 20k chelates positively charged cations by measuring force profiles in K_2SO_4. We found that the apparent surface potential was greatly reduced when divalent counterions were added to solution (results not shown).

(13) In the absence of *added* electrolyte, 20k PEG still adsorbed to the bilayer surface. However, we also measured a long-range, apparently electrostatic repulsion in these experiments. Conversely, PEG 20k did not adsorb to bare, uncoated mica substrates in the absence of added electrolyte (results not shown).

(14) Other reasons for a reduction in viscosity are shear thinning of the polymer solution at these intermediate separations, or an anomalous structural change of the polymer coils due to their interaction with the complexing ions in solution. Leibler, L.; Pezron, E.; Pincus, P. A. *Polymer* **1988**, *29*, 1105−1109.

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