Osmotic Properties of Poly(Ethylene Glycols): Quantitative Features of Brush and Bulk Scaling Laws

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ABSTRACT From glycosylated cell surfaces to sterically stabilized liposomes, polymers attached to membranes attract biological and therapeutic interest. Can the scaling laws of polymer "brushes" describe the physical properties of these coats? We delineate conditions where the Alexander-de Gennes theory of polymer brushes successfully fits the intermembrane distance versus applied osmotic stress data of Kenworthy et al. for poly(ethylene glycol)-grafted multilamellar liposomes. We establish that the polymer density and size in the brush must be high enough that, in a bulk solution of equivalent monomer density, the polymer osmotic pressure is independent of polymer molecular weight (the des Cloizeaux semidilute regime of bulk polymer solutions). The condition that attached polymers behave as semidilute bulk solutions offers a rigorous criterion for brush scaling-law behavior. There is a deep connection between the behaviors of semidilute polymer solutions in bulk and polymers grafted to a surface at a density such that neighbors pack to form a uniform brush. In this regime, two-parameter unconstrained fits of the Alexander-de Gennes brush scaling laws to the Kenworthy et al. data yield effective monomer lengths of 3.3–3.6 Å, which agree with structural predictions. The fitted distances between grafting sites are larger than expected from the nominal mole fraction of poly(ethylene glycol)-lipids; the chains apparently saturate the surface. Osmotic stress measurements can be used to estimate the actual densities of membrane-grafted polymers.

INTRODUCTION

Membrane surfaces decorated with end-grafted polymers are ubiquitous in biology (e.g., glycosylated cell surfaces such as the red blood cell glycocalyx). Organelles (e.g., microtubules) also possess polymeric hairs that are integral to their function (Sackett, 1995). A relatively well-defined example of a surface-attached polymeric coat is that of poly(ethylene glycol) (PEG), also known as poly(ethylene oxide). These polymer coatings attract strong interest because of their ability to shield surfaces from close interactions with other surfaces, including macromolecules. They thus provide steric stabilization to colloidal suspensions (Lasic and Martin, 1995), biocompatibility to medically implanted materials (Dumitriu, 2002), and ''stealth'' properties to intravenously injected liposomes. Such liposomes are in current therapeutic use as vehicles for in-vivo drug delivery (Lasic and Martin, 1995; Lasic and Papahadjopoulos, 1998).

It is commonly believed that to provide effective shielding of a surface from interactions with proteins in the bathing solution, the attached polymers must provide a surface layer of adequate coverage and thickness; i.e., they must approximate a surface ''brush'' (Szleifer, 1997). Polymers dilutely grafted to a surface are said to form ''mushrooms'' when the mean distance between grafting sites D is larger than the polymer size R_F (Flory radius), so that individual polymer chains remain separated and do not interact with each other. If D is decreased (higher grafting density) and/or R_F is increased (longer polymers) such that $D \simeq R_F$, then individual chains start to overlap and the polymers begin to interact. This overlap criterion defines the so-called mushroom-to-brush transition, and is commonly used to signify the beginning of brush-like behavior of the grafted polymer layer. A major point of this paper is that brush scaling laws are not applicable in this so-called weak overlap regime. Validity of brush scaling laws can be expected only for strong overlap, which is defined by a criterion different than the above.

The physicochemical characterization of a system of PEG chains end-grafted to the surface of a supporting lipid bilayer has biological and practical significance, but is also relevant to polymer physics (de Gennes, 1987). A well-tested scaling theory of the polymer brush, due to Alexander and de Gennes (AdG) exists and is currently well accepted (Alexander, 1977; de Gennes, 1987). It is thus important to ascertain whether, or under what conditions, the grafted PEG system adheres to established brush scaling laws.

To date, several investigators have addressed this issue by performing compression experiments in which two or more PEG-grafted bilayers are forced together, and force versus intermembrane distance is measured. The geometry of such an arrangement is illustrated in Fig. 1, where parameters pertinent to the theoretical analysis (see below) are also shown. For such data to be relevant to brush scaling laws, two criteria must be met: 1), the intermembrane forces must be dominated by interactions between the polymer chains, as opposed to electrostatic, van der Waals, hydration, or undulation forces; and 2), the grafted polymer chains must

Submitted April 22, 2002, and accepted for publication August 14, 2002.

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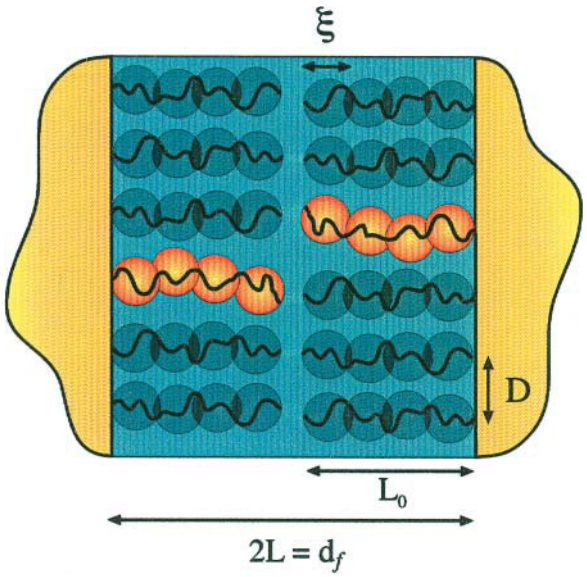

FIGURE 1 Schematic drawing of two apposed brush layers of surface-grafted PEG polymers in a multilamellar liposome. L_0 is the unperturbed thickness of the polymer brush, d_f is the "fluid spacing" or the surface-to-surface distance, $L \equiv d_f/2$, D is the distance between grafting sites, and ξ is the blob size of the polymer. Osmotic stress forces the surfaces together, leading to interaction and interpenetration of the brushes such that $L < L_0$. The yellow regions denote the lipid bilayers, and the blue region denotes the aqueous solution in between lamellae of the liposome.

exist in a strong brush conformation, as opposed to mushrooms or a weak brush-like state not far from the mushroom-to-brush transition.

Kuhl et al. (1994), using a surface force apparatus, measured force versus intermembrane distance for mica-supported bilayers whose apposed surfaces were composed of mixtures of distearoylphosphatidylethanolamine (DSPE) and PEG-grafted DSPE. Long-range repulsive forces were observed, and subtraction of the electrostatic component yielded repulsions attributable to interactions between the PEG surface layers. The densest conditions studied were PEG-2000 (45 monomers) at a nominal grafting density corresponding to 9 mol% PEG-PE.

Kenworthy et al. (1995a), using osmotic stress (OS), measured osmotic pressure versus intermembrane distance for multilamellar liposomes composed of mixtures of distearoylphosphatidylcholine (DSPC) and PEG-grafted DSPE. Clear evidence was given for interactions mediated by the PEG chains. Nominal densities of the grafted PEGs for liposomes made of Avanti lipids (Avanti Polar Lipids, Alabaster, AL) ranged up to 30 mol% PEG-2000 and 20 mol% PEG-5000 (113 monomers).

Another technique for measuring intermembrane force versus distance with PEG-grafted liposomes is that of micropipet manipulations (Needham et al., 1992; Evans et al., 1996). However, the conditions for these experiments have thus far not been amenable to brush scaling analysis.

In cases where results were analyzed in terms of the AdG brush scaling theory (Kuhl et al., 1994; Kenworthy et al., 1995a; Lasic, 1997), agreement between theory and experiment was not satisfactory. However, it has been noted (Szleifer, 1996) that most of the above data lies in a broad mushroom-to-brush transition region, and because scaling laws do not apply to this intermediate regime, quantitative fits are not to be expected. Szleifer (1996) has discussed the successful application of computer simulations and molecular theories to experimental data in the mushroom-to-brush transition regime. Although such approaches apply also in the strong-brush limit, they do not yield scaling laws and lack the robust predictions of scaling theory for semidilute solutions. Our interest here is to ascertain whether bulk and surface-grafted PEG systems can fulfill the criteria for analytical scaling theories, and if so, whether the scaling laws can be successfully applied.

The unanswered questions remain: can the grafted PEG layers on lipid membrane surfaces exist in a strong brush regime, and if so, do they obey brush scaling laws?

In this paper we first examine the relation between brush scaling laws and the behavior of polymer chains in bulk solution. Bulk PEG solutions are shown to exhibit scaling behavior under experimentally realizable conditions. We then examine the validity of AdG scaling laws when applied to PEG-grafted lipid bilayers. An operational criterion is presented for identifying the PEG brush scaling regime, and the range of validity of the scaling laws is shown to be more restrictive than often supposed. When applied to those data of Kenworthy et al. that satisfy the brush criterion, brush scaling laws are found to be valid. Further, fits to the data yield hitherto difficult-to-obtain information on the density of PEG grafts present on the bilayer surface. As the PEG-lipid mol fraction is increased, the fitted grafting densities plateau at values smaller than the nominal densities, indicative of surface saturation effects. The saturation mol fractions of PEG lipids in the bilayer are consistent with earlier estimates of this phenomenon.

RESULTS

When flexible polymers such as PEG are end-grafted to a surface, brush formation begins when $D \simeq R_F$, i.e., when the average distance between grafting sites D is comparable to the Flory radius $R_F = aN^{3/5}$, where a is the effective monomer length, and N is the number of monomers per polymer chain. However, it is important to realize that a brush is not fully developed until the surface-layer monomer density $\bar{\phi}$ is large enough (and chain overlap is strong enough) that a semidilute solution is formed. For a description of semidiluteness in a brush, see the Appendix and de Gennes (1979). It is important to emphasize that in a noncompressed brush, the celebrated linear relation between the brush thickness and the number of monomers per chain $L_0 = aN(a/D)^{2/3}$, as well as the molecular-

weight-independent relation $\bar{\phi}_0 \sim (a/D)^{4/3}$ between the spatially constant volume fraction of monomers and D, are both consequences of semidilute solution behavior in brushes (Alexander, 1977; de Gennes, 1987). We note that the Alexander-de Gennes model may be improved in several respects. For instance, one may self-consistently calculate an improved monomer density profile, as in Milner-Witten-Cates theory (Milner et al., 1988). Such improvements affect our force and osmotic pressure predictions to a very limited extent (Kenworthy et al., 1995a) and we shall ignore them here.

The natural way to test for possible semidilute solution behavior in PEG-lipid structures is to consider PEG under bulk conditions. We argue that flexible end-grafted PEG chains will form semidilute solutions only if bulk PEGs of comparable density form semidilute solutions. In bulk solutions, one may check for the desired property by determining whether the osmotic pressure Π is related to the bulk monomer volume fraction ϕ in the manner predicted by des Cloizeaux (de Gennes, 1979):

$$\Pi = \alpha(\mathsf{k_B T}/\mathsf{a}^3)\phi^{9/4}, \qquad (1)$$

where α is a constant $O(1)$. In Fig. 2 we plot the room-temperature bulk osmotic pressure as a function of bulk monomer volume fraction for PEG polymers of various molecular weights between 1000 and 20,000. The data were obtained from Rand (2002), which includes the data of Reid and Rand (1997).

Fig. 2 illustrates three points not previously addressed in studies of bulk PEG osmotic properties, including virial-expansion (Cohen and Highsmith, 1997) and excluded-volume (Reid and Rand, 1997) analyses of PEG osmotic pressures:

1. At high densities, the osmotic pressures of PEGs with molecular weight exceeding ~1500 Da indeed approach the des Cloizeaux result Eq. 1, if we take $a = 3.5$ Å (Kenworthy et al., 1995a) and fit $\alpha = 0.8$. Thus, sufficiently long PEG chains under bulk conditions indeed form semidilute solutions.
2. A crossover from ideal-gas behavior $\Pi = (k_B T/\, a^3)\phi/N$ at low volume fractions to des Cloizeaux behavior at high volume fractions takes place at higher concentrations the lower the molecular weight. Moreover, the chain-overlap condition $\phi = \phi^* \sim N^{-4/5}$ (de Gennes, 1979) does not provide a sufficient criterion for the attainment of des Cloizeaux behavior.
3. PEG-2000 solutions are in the scaling regime when the monomer volume fraction is larger than $\phi^{\#}_{2000} \simeq 0.15$, rather than the overlap volume fraction $\phi^{*}_{2000} \simeq 0.05$. Similarly, in view of the experimental uncertainty, PEG-5000 solutions are not in the scaling regime until the monomer volume fraction is larger than $\phi^{\#}_{5000} \simeq 0.07 - 0.09$, rather than $\phi^{*}_{5000} \simeq 0.02$.

Bulk-solution analysis of PEG data helps us establish whether AdG theory applies to stressed PEG-liposomes (Kuhl et al., 1994; Kenworthy et al., 1995a). Specifically, just before two brush-covered surfaces are brought into first contact, $\Pi \simeq 0$ and the thickness of the layers is $L \simeq L_0$. The monomer volume fraction in the brush is therefore $\bar{\phi}_0 \sim (a/D)^{4/3}$. If we assume $a = 3.5$ Å and use the relation $D \sim (A/f)^{1/2}$, where f is the mol fraction of PEG-lipids and A is the area per DSPC lipid = 48 Å^2 (Kenworthy et al.,

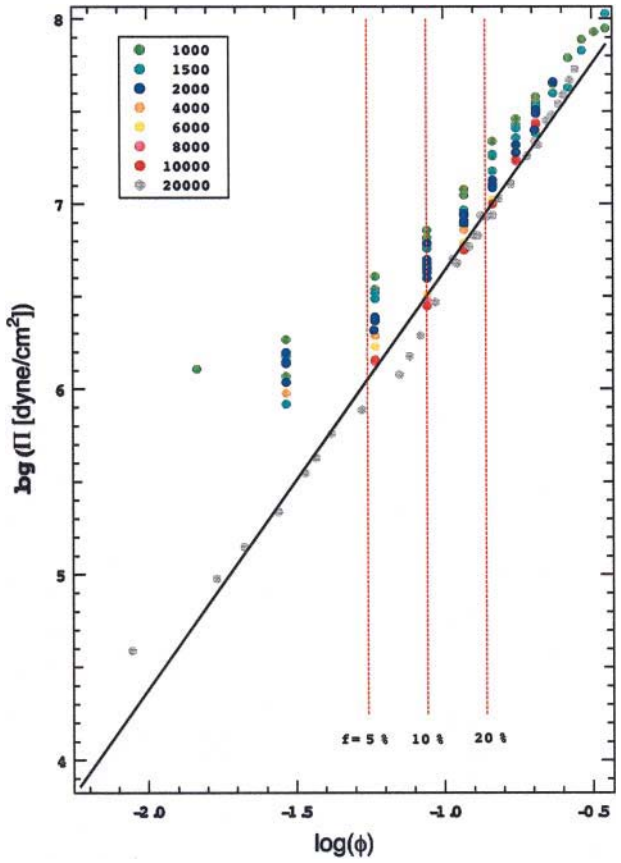

FIGURE 2 Room-temperature bulk osmotic pressure Π of various PEG polymers versus monomer volume fraction ϕ. The symbols are data obtained from Rand (2002) for PEGs of molecular weights 1000, 1500, 2000, 4000, 8000, 10,000, and 20,000 Da. The monomer volume fraction is $\phi \simeq 0.59 \times \mathsf{w}$, where $0 < \mathsf{w} < 0.5$ is the weight fraction of polymer (Rand, 2002). (In obtaining the relation between ϕ and w, we have assumed the specific density of PEG solutions to be 1 g/cm^3 (Hasse et al., 1995).) The solid line is the des Cloizeaux equation $\Pi = \alpha(\mathsf{k_B T}/\mathsf{a}^3)\phi^{9/4}$, with $\mathsf{a} = 3.5$ Å (Kenworthy et al., 1995a) and $\alpha = 0.8$. Note that at high ϕ the data converge to a universal straight line independent of molecular weight, in agreement with the des Cloizeaux prediction. For reference, the vertical dashed lines are the molecular-weight-independent monomer volume fractions in an uncompressed brush $\bar{\phi}_0 = (\mathsf{a}/\mathsf{D})^{4/3}$ for PEG-lipid mol fractions $\mathsf{f} = 0.05$, 0.1, and 0.2 if $\mathsf{a} = 3.5$ Å and $\mathsf{D} = (\mathsf{A}/\mathsf{f})^{1/2}$, where $\mathsf{A} = 48$ Å^2 (Kenworthy et al., 1995a). A surface-grafted PEG layer is in the brush scaling regime if the free-polymer bulk Π lies on the des Cloizeaux line at monomer volume fraction ϕ equal to $\bar{\phi}$ in the surface layer (vertical dashed lines). Thus, at a 10% grafting density, a PEG-6000 layer is in the brush scaling regime, but a PEG-1500 layer is not. See text and Appendix for details. (Enlargements of this figure are available upon request.)

1995a), we find that monomer solutions of DSPC:PEG-2000 reach the critical value $\phi^{\#}_{2000} \simeq 0.15$ at PEG-lipid mol fraction $f^{\#}_{2000} \simeq 0.23$. Similarly, solutions of DSPC:PEG-5000 reach the critical value $\phi^{\#}_{5000} \simeq 0.07 - 0.09$ at mol fraction $f^{\#}_{5000} \simeq 0.07 - 0.10$.

In Fig. 3 we display the Avanti DSPC:PEG-5000 OS data reported by Kenworthy et al. (1995a). We focus on the behavior at the highest coverages corresponding nominally to $f = 0.1$ and 0.2, where bulk analysis leads us to believe that AdG theory may well apply.

According to AdG theory (Alexander, 1977; de Gennes, 1987) (see Appendix), the formation of a brush is a compromise between excluded-volume repulsions, which lead to an osmotic contribution similar to the des Cloizeaux result, and grafting constraints that are responsible for entropic elastic tensions. The expression for the osmotic pressure reflects the difference between these effects:

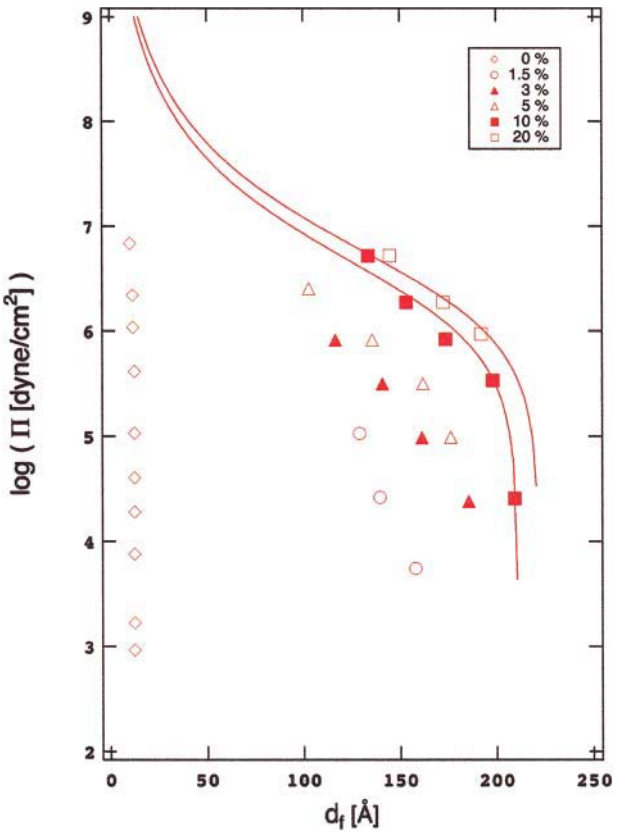

FIGURE 3 Osmotic pressure Π versus fluid spacing d_f between apposing PEG-grafted bilayers. The symbols are data from Kenworthy et al. (1995a) for Avanti DSPC:PEG-5000 complexes with nominal grafting mol fractions $f = 0$, 0.015, 0.03, 0.05, 0.1, and 0.2. The solid curves are fits to the $f = 0.1$ and 0.2 data using Eqs. 2 and 3, where $L = d_f/2$. Two-parameter unconstrained fits of the AdG expression for osmotic pressure versus fluid spacing yield nearly constant effective monomer lengths $a_{0.1} = 3.56 \pm 0.07$ Å and $a_{0.2} = 3.30 \pm 0.15$ Å. These numbers agree well with structural values reported in the literature (Kenworthy et al., 1995a), lending veracity to the applicability of AdG theory to these data. The $f = 0.015$, 0.03, 0.05 data do not meet the brush criterion (see Fig. 2), and thus AdG theory is not applicable. See text for details.

$$\Pi(L) = \alpha \frac{k_B T}{D^3} \left[\left(\frac{L_0}{L} \right)^{9/4} - \left(\frac{L}{L_0} \right)^{3/4} \right], \tag{2}$$

where

$$L_0 = aN \left(\frac{a}{D} \right)^{2/3}. \tag{3}$$

We conjecture that $\alpha = 0.8$ as for bulk PEG solutions (see Eq. 1).

One may test AdG theory by substituting $D(L_0, a)$ from Eq. 3 into the expression $\Pi(L)$ in Eq. 2 and performing unconstrained fits with the two free parameters a and L_0. The solid lines in Fig. 3 are the results of such fits. The quality of the procedure is gauged by the degree to which roughly constant and ''reasonable'' values of a are obtained. We indeed get nearly constant a's close to the values cited in the literature (Kenworthy et al., 1995a): $a_{0.1} = 3.56$ Å and $a_{0.2} = 3.30$ Å. The fitted values for the brush thicknesses are $L_0 = 105$ Å for $f = 0.1$, and $L_0 = 109$ Å for $f = 0.2$, respectively. The uncompressed brush thickness L_0 varies as expected: the higher the coverage, the thicker the brush. However, the grafting densities implied by these fits are systematically lower than the nominal values. Assuming $f = A/D^2$ with $A = 48$ Å^2 and D from Eq. 3 with $a = 3.5$ Å, we find $f_{0.1} = 0.07$ and $f_{0.2} = 0.08$.

In summary, good fits producing nearly constant (unconstrained) values of a, with fitted f's lower than nominal values but still within the semidilute regime (f's $\geq f^{\#}_{5000}$), indicate that scaling analysis of the PEG-5000 data of Kenworthy et al. is valid and self-consistent. Saturation effects in these dense brushes are indicated by lower than nominal fitted values of f.

For DSPC:PEG-2000, AdG analysis shows similar saturation effects. In the case of 30 mol% PEG-lipid, fits yield $f_{0.3} < f^{\#}_{2000}$, thus the monomer density is below the onset of semidilute behavior. Therefore in this case the fitting procedure is not self-consistent. AdG theory is even less applicable for smaller mol fractions of PEG-2000 lipids.

DISCUSSION

This paper emphasizes that brush and bulk scaling laws are related and illustrates how the relation yields information about brush formation in PEG-grafted liposomes. On one hand, a scaling analysis of bulk osmotic pressure data for PEGs of various molecular weights establishes criteria stronger than the popular overlap criterion $D \simeq R_F$ (Kuhl et al., 1994; Kenworthy et al., 1995a; Belsito et al., 2000) for invoking the AdG theory of brush behavior: In order to invoke Eq. 2, it must be established that the polymer size and density in the compressed brush are in a regime where bulk des Cloizeaux scaling applies. Consequently, we are forced to forgo AdG analysis for PEG-grafted liposomes containing PEGs of molecular weight 2000 Da or less. On the other

hand, having established that semidilute scaling regimes are within reach for long bulk PEG polymers, we are increasingly confident that AdG theory (or improvements thereof—see note on the Alexander-de Gennes model in Results) is the correct framework for analyzing densely grafted liposomes containing PEGs of molecular weight greater than ~2000 Da.

Other experimental studies lend support to the view that long polymers end-grafted to nonadsorbing substrates under good solvent conditions have bulk scaling behavior as predicted by des Cloizeaux and brush scaling behavior as described by Alexander and de Gennes (Auroy et al., 1991; Taunton et al., 1990). Our analysis illustrates that brush formation with long water-soluble polymers is fundamentally no different than brush formation with synthetic polymers in organic solvent.

The fact that our two-parameter fits to the AdG expression (Eqs. 2 and 3) are of such quality that they yield unbiased prediction of fairly constant effective monomer lengths $a \simeq 3.5\,\text{Å}$, in good agreement with structural values (Kenworthy et al., 1995a), leads us to conjecture that OS measurements offer a method for nontrivial, semiquantitative determinations of surface structure for PEG-grafted liposomes. Hitherto few physicochemical methods (Belsito et al., 2000; Montesano et al., 2001) have been invoked to refine estimates of grafting densities beyond nominal values. Additional methods are desirable, not the least because of the technological importance of PEG-grafted liposomes.

With scaling analysis of OS data, reliable determination of the brush grafting density is possible, assuming of course that reliable OS data can be obtained in the semidilute regime. From the grafting density, an estimate of the monomer density in the brush, $\bar{\phi}_0$, can be obtained. Assuming the area per lipid, A, to be well determined, the mol fraction of PEG-lipids in a brush, $f = f(\bar{\phi}_0)$, can be determined too. Several values have been reported for the area per lipid of DSPC (Kenworthy et al., 1995a; Rand and Parsegian, 1989; Lis et al., 1982). Irrespective of which value is used in the calculations, we predict that densely grafted DPSC:PEG-lipid liposomes are subject to surface saturation effects. For example, for DSPC:PEG-5000 with nominal mol fractions 0.1 and 0.2, the difference between the fitted grafting densities is small. Assuming $A = 48\,\text{Å}^2$ (Kenworthy et al., 1995a), we find $f_{0.1} = 0.07$ and $f_{0.2} = 0.08$. The origin of such saturation effects is not yet well understood.

On theoretical grounds (Hristova and Needham, 1995), one expects that addition of extra PEG-lipids will cause an increase in lateral pressure until formation of nonbilayer structures such as micelles is favored. In PEG-grafted liposomes with dipalmitoylphosphatidylcholine (DPPC) as the host lipid, this mechanism seems to be active (Belsito et al., 2000; Montesano et al., 2001). In DSPC:PEG liposomes below the chain-melting temperature, the situation is more complicated. For DSPC:PEG-5000, Kenworthy et al. (1995b) suggest that before micelle formation sets in, a moderate increase in PEG-lipid content is accompanied by a transformation from an $L_{\beta'}$-like phase to an L_{β}-like phase. Interestingly, the transformation between these phases is argued to set in at mol fractions of PEG-lipid corresponding approximately to the saturation limit calculated here, i.e., $f_{5000} \simeq 0.1$.

CONCLUSIONS

Sufficiently long PEG polymers in bulk solution can form semidilute solutions with osmotic properties as foreseen by des Cloizeaux (de Gennes, 1979). Sufficiently dense and thick ''brushes'' of PEG-lipids end-grafted to lipid bilayers are therefore expected to behave as confined semidilute solutions with a scaling structure as predicted by Alexander and de Gennes (Alexander, 1977; de Gennes, 1987). The approach to bulk semidilute behavior is slow for PEGs of molecular weight less than several thousand Da. When attached to lipids, those PEGs will form brushes satisfying AdG theory only if they are rather dense with grafting densities exceeding $f^{\#} \simeq 0.2$. In practice this condition may be difficult to realize. Of all the data reported in the literature (Kuhl et al., 1994; Kenworthy et al., 1995a), only the osmotic stress data for DSPC:PEG-5000 with nominal grafting densities in excess of $f^{\#} \simeq 0.1$ approximately satisfy the AdG theory. Two-parameter unconstrained fits to these data using the AdG expression for osmotic pressure versus bilayer separation yield good fits with effective monomer length $a \simeq 3.5\,\text{Å}$ in agreement with structural values. The coverages inferred from the fits are lower than the nominal coverages, an indication of surface saturation effects that are now beginning to be understood. We conjecture that osmotic-stress measurements provide a method for semiquantitative structure determinations of PEG-grafted-liposome surfaces.

A quantitative characterization of brush scaling behavior and structure relies on a precise identification of semidiluteness that is more rigorous than the simple, and often-used, chain-overlap criterion, $D \simeq R_F$.

APPENDIX

Isolated, noncompressed brush (de Gennes, 1987, 1979): In a fully developed noncompressed brush, chain overlap is so strong that a semidilute solution of spatially-constant monomer volume fraction $\bar{\phi}_0$ is formed (however, see note on the AdG model in Results). We recall that under semidilute solution conditions: 1), the relevant degree of freedom is a ''blob'' characterized by its size $\xi(\bar{\phi}_0)$ and its free energy k_BT; 2), the gas of blobs is noninteracting, but the chains inside a blob interact solely via excluded-volume repulsions, so the number of monomers g_ξ inside a blob is related to blob size by the Flory relation $\xi(\bar{\phi}_0) = a g_\xi^{3/5}$, where a is the effective monomer length; and 3), most physical properties become molecular-weight independent.

In noncompressed brushes, the blob size is determined by the distance between grafting sites, $\xi(\bar{\phi}_0) \sim D$. At the same time, molecular-weight independence of physical properties in semidilute solutions implies that $g_\xi = g_D \sim \bar{\phi}_0^{-5/4}$, and $\xi(\bar{\phi}_0) \sim a\bar{\phi}_0^{-3/4}$, as in semidilute bulk solutions. It

follows that $\bar{\phi}_0 \sim (a/D)^{4/3}$. Polymer chains in the brush form strings of blobs. The length of a noncompressed string is the blob size times the number of blobs, i.e., $L_0 \sim D \times (N/\, g_D) \sim aN(a/D)^{2/3}$. This well-known linear relation between L_0 and N reflects the strong stretching of chains in a brush and is largely a consequence of semidilute solution behavior in the brush.

Compressed brush: It is instructive to view the formation of a brush as a compromise between excluded-volume monomer repulsions, which lead to an osmotic contribution, and confinement effects (due to the grafts) that are responsible for entropic elastic tensions. In the absence of osmotic stress, these effects balance each other. When subjected to compression, apposing brushes begin to overlap, the brush thickness L decreases, and the monomer density in the brush increases: $\bar{\phi}_L = \bar{\phi}_0 \times (L_0/L)$. The osmotic stress generates an imbalance between osmotic and elastic terms that may be described as compression of a string of blobs. In fact, the resulting osmotic pressure can be derived from the free energy per chain $F_c = F_{os} + F_e$, where the osmotic term is k_BT per blob times the number of blobs, as in bulk semidilute solutions. Thus $F_{os} \sim k_BT \times (N/g_L)$, where g_L is the number of monomers per blob in the compressed brush. Invoking the Alexander condition $g_L \sim \bar{\phi}_L^{-5/4}$ yields $F_{os} \sim k_BTN\bar{\phi}_L^{5/4}$. The elastic term is a Flory-type entropic elastic free energy for an ideal random walk of blobs, $F_e \sim k_BTL^2/R^2(\bar{\phi}_L)$, with $R^2(\bar{\phi}_L)$ given by the blob size squared times the number of blobs, $R^2(\bar{\phi}_L) = \xi^2(\bar{\phi}_L) \times (N/g_L)$. From the Flory relation $\xi(\bar{\phi}_L) = ag_L^{3/5}$, it follows $\xi(\bar{\phi}_L) \sim a\bar{\phi}_L^{-3/4}$ (the relation $\xi(\bar{\phi}_L) \sim D$ is not valid), and invoking the above expression $L = L_0\phi_0/\bar{\phi}_L$ yields $F_e \sim k_BTN(a/D)^4\bar{\phi}_L^{-7/4}$. The osmotic pressure $\Pi = \bar{\phi}_L^2\partial_{\bar{\phi}_L}F_c/(Na^3)$ (de Gennes, 1979) can now be derived in the form of Eq. 2.

Relation to bulk solution behavior: For free polymer in solution, $\bar{\phi}_L \longrightarrow \phi$, and the free energy contains no elastic restoring term. From the blob expression $F_{os} \sim k_BTN\phi^{5/4}$, we readily infer the des Cloizeaux expression Eq. 1 for bulk polymers in the semidilute regime. It is important to note that the validity of Eq. 1 for bulk polymers is a necessary condition for the validity of Eq. 2 for the compressed brush. To invoke Eq. 2, it must be established that the polymer size and density in the compressed brush are in a regime where bulk des Cloizeaux scaling applies.

We thank Dr. A. Kenworthy for valuable discussions. P.L.H. thanks Prof. F. Pincus for valuable comments.

The Center for Biomembrane Physics (MEMPHYS) of the University of Southern Denmark is funded by the Danish Natural Science Research Council.

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