Interaction of Poly(ethylene-glycols) with Air-Water Interfaces and Lipid Monolayers: Investigations on Surface Pressure and Surface Potential

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ABSTRACT We have characterized the surface activity of different-sized poly(ethylene-glycols) (PEG; M_r 200–100,000 Da) in the presence or absence of lipid monolayers and over a wide range of bulk PEG concentrations (10^{-8}-10% w/v). Measurements of the surface potential and surface pressure demonstrate that PEGs interact with the air-water and lipid-water interfaces. Without lipid, PEG added either to the subphase or to the air-water interface forms relatively stable monolayers. Except for very low molecular weight polymers (PEGs < 1000 Da), low concentrations of PEG in the subphase (between 10^{-5} and 10^{-4}% w/v) increase the surface potential from zero (with respect to the potential of a pure air-water interface) to a plateau value of ~440 mV. At much higher polymer concentrations, $>10^{-1}$% (w/v), depending on the molecular weight of the PEG and corresponding to the concentration at which the polymers in solution are likely to overlap, the surface potential decreases. High concentrations of PEG in the subphase cause a similar decrease in the surface potential of densely packed lipid monolayers spread from either diphytanoyl phosphatidylcholine (DPhPC), dipalmitoyl phosphatidylcholine (DPPC), or dioleoyl phosphatidylserine (DOPS). Adding PEG as a monolayer at the air-water interface also affects the surface activity of DPhPC or DPPC monolayers. At low lipid concentration, the surface pressure and potential are determined by the polymer. For intermediate lipid concentrations, the surface pressure-area and surface potential-area isotherms show that the effects due to lipid and PEG are not always additive and that the polymer's effect is distinct for the two lipids. When PEG-lipid-mixed monolayers are compressed to surface pressures greater than the collapse pressure for a PEG monolayer, the surface pressure-area and surface potential-area isotherms approach that of the lipid alone, suggesting that for this experimental condition PEG is expelled from the interface.

INTRODUCTION

Poly(ethylene glycol) is a linear polymer with extensive industrial, basic research, and biotechnological applications. It is used to precipitate water-soluble proteins (Albertson et al., 1986) and to initiate cell-cell fusion, which is a necessary step for the production of hybridoma cells (Ahkong and Lucy, 1986). Recently, interest in PEG's usefulness in biomedical applications has increased, particularly because it is nontoxic. For example, attaching PEG to surfaces exposed to biological materials renders them relatively inert and less likely to induce blood clots (Harris, 1992). In addition, when covalently linked to the headgroup of lipids, PEGs can shield the surface from protein adsorption, thereby imparting a "stealth-like" property to the surface (Harris, 1992). Also, PEG covalently attached to lipids extends the longevity of liposomes, which is beneficial for some methods of drug delivery (Woodle and Lasic, 1993).

Nonelectrolyte water-soluble polymers such as PEG are an important tool for biophysical studies of macromolecules (e.g., DNA, lipids, and proteins) because they commonly are used to establish a colloid osmotic pressure that forces molecules into close proximity (Podgornik et al., 1994; Rand and Parsegian, 1989). In recent studies, a variety of such polymers was used to estimate several physical properties of ionic channels. For example, polymers that do not permeate the channel were used to estimate the change in the pore volume occurring upon channel gating (e.g., Zimmerberg and Parsegian, 1986). Also, a sufficient number of low molecular weight PEGs are commercially available with sizes that are convenient for determining the access resistance and pore diameter of ion channels (Scherer, 1971; Kuga, 1981; Krasilnikov et al., 1991; Vodyanoy and Bezrukov, 1992; Vodyanoy et al., 1993; Kasianowicz et al., 1994; Bezrukov et al., 1994). Implicit in the latter studies is the assumption that the polymer does not interact with lipids or channels.

PEG has also been used in the study of cell and liposome fusion (e.g., Wilschut and Hoekstra, 1991; Li and Hui, 1994), but the underlying mechanisms by which the polymer induces this effect are still a matter of debate. The study of liposome fusion in presence of PEG has demonstrated the complexity of this process (Saez et al., 1982; Aldwinckle et al., 1982; Tilcock and Fisher, 1982; Boni et al., 1984, 1987; Burgess et al., 1992). The fusion yield appears to depend on the size of the liposome (Burgess et al., 1991; Lentz et al., 1992) and the presence of other "fusogenic"

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Abbreviations used: PEG, poly(ethylene glycol); DOPS, dioleoyl phosphatidylserine; PEO, poly(ethylene oxide); DPhPC, diphytanoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; *P-A*, surface pressure-area; ΔV-*A*, surface potential-area; *A*, molecular area; MES, 2-morpholinoethanesulfonic acid (monohydrate); MW, molecular weight.

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compounds (Massenburg and Lentz, 1993). A concentration of PEG greater than a critical value induces fusion (MacDonald, 1985; Parente and Lentz, 1986; Massenburg and Lentz, 1993; Viguera et al., 1993), whereas lower concentrations lead only to aggregation of the individual liposomes (Parente and Lentz, 1986; Viguera et al., 1993). Interestingly, PEG can induce fusion whether present with the particles or separated from them by a dialysis bag (MacDonald, 1985). PEG induces leakage of liposomes (Viguera et al., 1993; Massenburg and Lentz, 1993), and studies on large unilamellar vesicles suggest that the leakage is caused by the rupture of the liposomes and is not due to small (aqueous) pores (Massenburg and Lentz, 1993). Several mechanisms of how PEG causes the primary molecular event of fusion were proposed (for a short review, see Lentz, 1994). For example, the change in the surface potential caused by PEG was suggested to be a determining factor in cell-cell fusion (Maggio et al., 1976). Others found that fusion correlates with the change in dielectric constant caused by PEG (Ohki and Arnold, 1990). Another model suggests that the polymer binds water and dehydrates the membrane, which presumably causes the aggregation of cells and subsequently induces membrane fusion (Arnold et al., 1990).

PEG has several interesting physical and chemical characteristics. For example, at room temperature, it is completely soluble in aqueous solution and in chloroform (Bailey and Koleske, 1976). In contrast, a similar polymer constructed from methylene instead of ethylene monomers is insoluble in water. At elevated temperature, PEG and water partially phase-separate (Malcolm and Rowlinson, 1957; Saeki et al., 1976). PEG forms complexes with metallic ions or changes the ion activity (Bailey and Koleske, 1976; Gawrisch et al., 1987). In earlier studies, PEG was shown to degrade under ultraviolet light under the action of acids or metal ions and to form hyperoxides (Bailey and Koleske, 1976; Harris, 1993). Conformational studies of PEG in bulk aqueous solutions were done using light scattering, NMR, and infrared and Raman spectroscopy. These techniques show that PEG binds ~2–3 water molecules per ethylene glycol monomer (Bailey and Koleske, 1976; Polik and Burchard, 1983; Wesseling et al., 1991; Harris, 1993). NMR measurements also demonstrate that low molecular weight PEGs have a zigzag conformation, whereas higher molecular weight variants form random coils (Bailey and Koleske, 1976; Harris, 1993). Measurement of the static dielectric constant of PEG in aqueous solution showed that increasing the PEG concentration causes first a slight increase and then a decrease to a value of 50 at ~50 wt% PEG (Arnold et al., 1985).

PEGs spread at an air-water interface form stable monolayers, and the collapse pressure of the films increases slightly with the polymer molecular weight (Glass, 1968; Shuler and Zisman, 1970; Sauer et al., 1987; Nitsch et al., 1987; Kawaguchi et al., 1988; Kuzmenka and Granick, 1988; VanOss and Good, 1990, Sauer and Dee, 1994). Careful studies on PEG 90,000 monolayers suggested that the polymer is oriented at the air-water interface and becomes expelled from the interface near its collapse pressure (Shuler and Zisman, 1970). Ellipsometric studies of the air/water interface on PEG monolayers show that the PEG is present in a narrow layer (0.34–1 nm) near the interface (Kawaguchi et al., 1988; Sauer et al., 1989). However, little is known about the conformation of PEG at the air-water or lipid-water interfaces.

Much less is known about the effect of these polymers on the properties of lipid monolayers. In an earlier report, it was shown that adding PEG to the subphase changes the surface potential of lipid monolayers (Maggio et al., 1976) (see Results and Discussion). PEG also shifts the phase transition temperature of lipids (Tilcock and Fisher, 1979), presumably by changing the solvation property of water. A reduction in the mobility of the lipid molecules upon addition of PEG was observed (Ohno et al., 1981; Lehtonen and Kinnunen, 1994). ^{2}H-NMR investigations have shown that PEG dehydrates the lipid membrane and that the NMR spectra resemble those of reduced water concentration (Arnold et al., 1983; Arnold and Gawrisch, 1993).

In this study, we extended the measurements of PEG's surface activity and determined the effect of the polymer on the surface pressure and surface potential at the air-water and air-lipid-water interfaces over a wide range of polymer concentration and molecular weight. We also studied the effect of adding PEG to the air-water and air-lipid-water interfaces.

MATERIALS AND METHODS

Different PEGs with molecular weight 200, 400, 600, 1000, 2000, 3000, 8000, 20,000, and 35,000 were purchased from Fluka (Neu-Ulm, Germany) and with molecular weight 600, 2000, and 100,000 from Aldrich (Steinheim, Germany). Concentrated stock solutions of PEG were made by dissolving a known amount of polymer in a fixed volume of buffer solution (% w/v). Unless otherwise noted, the buffer contained 0.15 M NaCl (Merck, Darmstadt, Germany), 2 mM MES (Merck), titrated to pH 6.0 with 1 M NaOH. The water was purified with a Milli-Q system (Millipore Corp., Bedford, MA).

To determine whether the effects of PEG on surface activity were due to the polymer and/or trace contaminants, we purified several different molecular weight samples of PEG (MW 2000, 35,000, and 100,000) according to a standard procedure in which the polymer is solubilized in chloroform, precipitated in diether and then subjected to exhaustive dialysis against purified water (Honda et al., 1981). The dialysed PEG was subsequently lyophilized. We observed no difference between the surface active properties of the purified and unpurified material. In addition, PEGs of the same molecular weight purchased from different suppliers gave identical results.

We measured the surface pressure and surface potential caused by PEGs in the presence and absence of lipid monolayers in two different types of troughs. The first trough (fixed surface area = 120 cm^2, volume = 300 ml), which was milled from virgin Teflon, contained a hole in its side through which small aliquots of concentrated stock PEG solutions were introduced to the subphase. A Teflon-coated stir bar located next to the hole ensured rapid dispersal of the PEG solutions (in a separate control experiment, adding a dye to the polymer stock solution confirmed that the subphase in the trough was made uniform in a few seconds). The second unit was a Lauda FW 1 monolayer trough (MGW Lauda, Germany). It should be noted that PEG apparently adsorbs to Teflon. Extensive rinsing with water was usually required to remove the polymer from the chamber. Changing

the pH (pH 4 and 11) had no effect on the surface pressure and surface potential induced by PEG 2000.

The surface pressure was measured using the Wilhelmy plate method (Allan, 1958; Gaines, 1966) using a plate made from a 1-cm-perimeter piece of Whatman #1 filter paper (Maidstone, UK) or of platinum. The surface potential was measured using the Kelvin vibrating plate method (Gaines, 1966) using a 2-cm-diameter, gold-plated disk electrode, which vibrated at 416 Hz using a laboratory-built lock-in amplifier (Bürner et al., 1994) The gold-plated disk electrode was located <1 mm above the air-water interface. The surface potential was referenced to a Ag/AgCl electrode in the subphase. Monolayers of dipalmitoyl phosphatidylcholine (DPPC), diphytanoyl phosphatidylcholine (DPhPC), or dioleoyl phosphatidylserine (DOPS) (all obtained from Avanti Polar Lipids, Alabaster, AL) were spread at the air-water interface from a 1:1 (v/v) benzene/ethanol solution (Merck). The drift of the surface potential was ~15 mV/h. The drift due to water evaporation was compensated by readjusting the height of the vibrating plate. For each molecular weight of PEG, the concentration was increased starting from that which caused a detectable change in the surface pressure-area (*P-A*) or surface potential-area (ΔV-*A*) isotherms.

Lipid monolayers were also spread at the air-water interface by adding 40–60 μl of 0.6–0.9 mg/ml DPhPC or DPPC in a 1:1 (v/v) benzene/ethanol solution to the Lauda monolayer trough. PEG was added to the surface from a 0.05% (w/v) chloroformic solution. The PEG and lipids were added using Hamilton syringes (Hamilton, Bonaduz, Switzerland). The initial surface area typically was 720 cm^2, and the absolute number of lipid molecules added to the trough was ~3.5×10^{16}. We waited 10 min for the solvents to evaporate. Unless otherwise indicated, the aqueous subphase contained 800 ml of buffer (0.15 M NaCl, 2 mM MES, pH 6.0). Before each experiment, the trough was cleaned with acetone and then rinsed extensively with deionized water. Spreading PEG onto the surface was performed carefully because it is more difficult to do than spreading a lipid monolayer. This is probably due to the high solubility of PEG in the aqueous phase. PEG also seems to facilitate the loss of lipids into the subphase, especially if the PEG-containing solution is added too rapidly or if the drops of spreading solution are too large.

P-A and ΔV-*A* isotherms were measured by slowly compressing a lipid monolayer at a rate of ~1.7 cm/min (25 cm^2/min). In several cases, we compressed a monolayer to a surface pressure just below the collapse pressure and then expanded it slowly. The films typically exhibited minimal hysteresis, depending on the surface pressure at which the expansion began. The surface pressure was measured using the horizontal force balance provided with the Lauda trough, and the surface potential was measured as described above. Both the surface pressure and surface potential signals were digitized using a 12-bit A/D board (BMC, Puchheim, Germany) installed in an IBM-compatible PC. We typically averaged the results of at least three experiments.

RESULTS

Effect of PEG on the properties of the air-water interface

We determined the surface activity of PEG in the absence of lipid when the polymer was either added to the subphase or spread at the air-water interface. We discuss first the results of adding polymer to the subphase.

Fig. 1 illustrates the change in the surface potential caused by adding different-sized PEGs to the subphase. For the sake of clarity, we only show here the results obtained with PEG 200 (*stars*), 2000 (*triangles*), and 100,000 (*circles*). A variety of other molecular weight PEGs (see Materials and Methods) produce results that are consistent with the data in Fig. 1. Adding polymer to the subphase causes three distinct regions of PEG surface activity as defined by the change in the surface potential with respect to that of

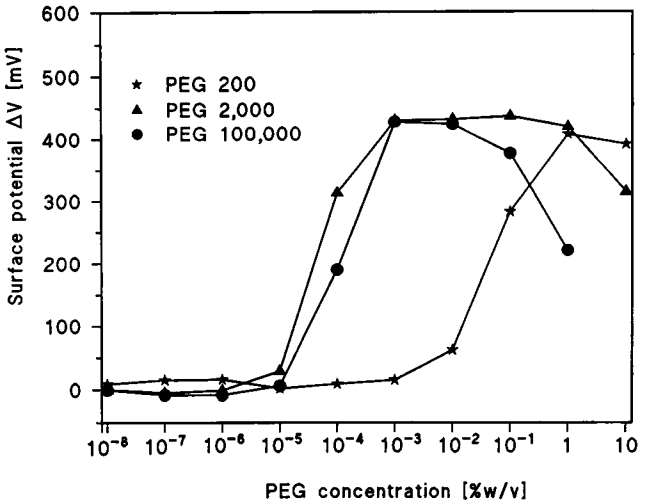

FIGURE 1 The change in the surface potential, ΔV, at the air-water interface induced by poly(ethylene-glycol). PEG with molecular weight of 200 (★), 2000 (▲), or 100,000 (●) was added to the subphase. The change in the surface potential is referenced to the air-water interface. The data points represent the mean values of at least three different measurements; the SD was less than ±15 mV in each case. The subphase initially contained 300 ml of 0.15 M NaCl, 2 mmol of MES, pH 6.0, T = 20°C.

either pure water or buffer solutions. First, PEG concentrations $<10^{-5}$% (w/v) cause virtually no change in the surface potential. Second, intermediate concentrations of PEG raise the surface potential to a maximum (or plateau) value $\Delta V \sim 440$ mV. This plateau value is independent of the PEG molecular weight. For intermediate and high molecular weight polymer ($M_r > 1000$ Da), 10^{-3}% (w/v) PEG is sufficient to achieve the maximum value of the potential. For lower molecular weight PEGs (e.g., PEG 200), the plateau value is reached only at much higher polymer concentrations (e.g. 1% w/v). Third, high concentrations of intermediate and high molecular weight PEGs (MW > 1000) cause a substantial decrease in the surface potential. Note further that higher molecular weight PEGs require a lower polymer concentration to initiate the decrease in the potential from its maximum value.

The decrease in the surface potential at high concentration of PEG occurs whether PEG is added to the subphase from highly concentrated (30% w/v) or more dilute (10^{-1}% w/v) stock solutions (data not shown). Also, diluting a subphase that initially contains a highly concentrated (10% w/v) PEG 20,000 buffer with PEG-free buffer to a final concentration of 0.02% (w/v) PEG causes the surface potential to increase by ~80 mV. (Similar effects were observed for PEGs 2000, 35,000, and 100,000 MW. However, the rise was always less than that anticipated from the results shown in Fig. 1. This may be due to incomplete mixing of the PEG in the subphase and the buffer that was subsequently added to the trough.)

The equilibration of bulk phase PEG with the air-water interface and the surfaces of the monolayer trough is somewhat complicated, especially at low polymer concentra-

tions. For example, carefully aspirating part of the surface of a 10^{-3}% (w/v) PEG 2000 solution causes a substantial decrease of the surface potential from the plateau value. Replacing the subphase containing PEG, originally at a sufficiently high concentration to cause the surface potential to reach its plateau value, with a PEG-free solution causes no significant variation in the surface potential. This result suggests that some of the PEG in solution is kinetically trapped at the air-water interface (see below). PEG's negligible surface activity at low polymer concentrations (i.e., $<10^{-5}$% (w/v); see Fig. 1) is probably due to the adsorption of PEG to the Teflon trough. This assumption is supported by the results of two control experiments. First, replacing the subphase by continuously injecting new PEG-buffer solution of the same low concentration (e.g., 10^{-4}% w/v) caused a visible increase of the surface potential after a while. Second, rinsing the Teflon trough several times with distilled water after an experiment in which high PEG concentrations were used and subsequently adding buffer to the trough resulted in an unusually high surface potential. This residual potential was never observed if the trough was cleaned extensively with distilled water and acetone.

Others have shown that PEG added to the air-water interface can form surprisingly stable films (e.g., Glass, 1968; Nitsch et al., 1987). We therefore characterized the surface pressure- and surface potential-area isotherms for PEG monolayers. The films are formed by carefully adding PEG to the surface from stock solutions dissolved in chloroform. After spreading the polymer, a movable barrier is used to compress the film. Fig. 2 *A* demonstrates the increase of the surface pressure accompanying the decrease of the surface area per polymer monomer. Compressing the monolayer to areas ~0.4–0.6 nm^2 per monomer causes a smooth increase of the surface pressure. Compressing the film to areas <0.15 nm^2 per monomer causes no further increase of the surface pressure. The area per PEG monomer at the collapse point was almost independent of the polymer molecular weight. However, the collapse pressure increases by a factor of two when PEG 100,000 is used instead of PEG 2000.

Fig. 2 *B* shows that compressing high molecular weight PEG (e.g., PEG 35,000 or 100,000) surface films increases the surface potential to a plateau value of $\Delta V \sim 450$ mV, which is virtually identical to the maximum value caused by adding PEG to the subphase (Fig. 1). However, the plateau value for small PEGs (e.g., 2000 MW PEG) was slightly smaller than that observed in the previous experiment in which the polymer was injected into the subphase (Fig. 1, *triangles*).

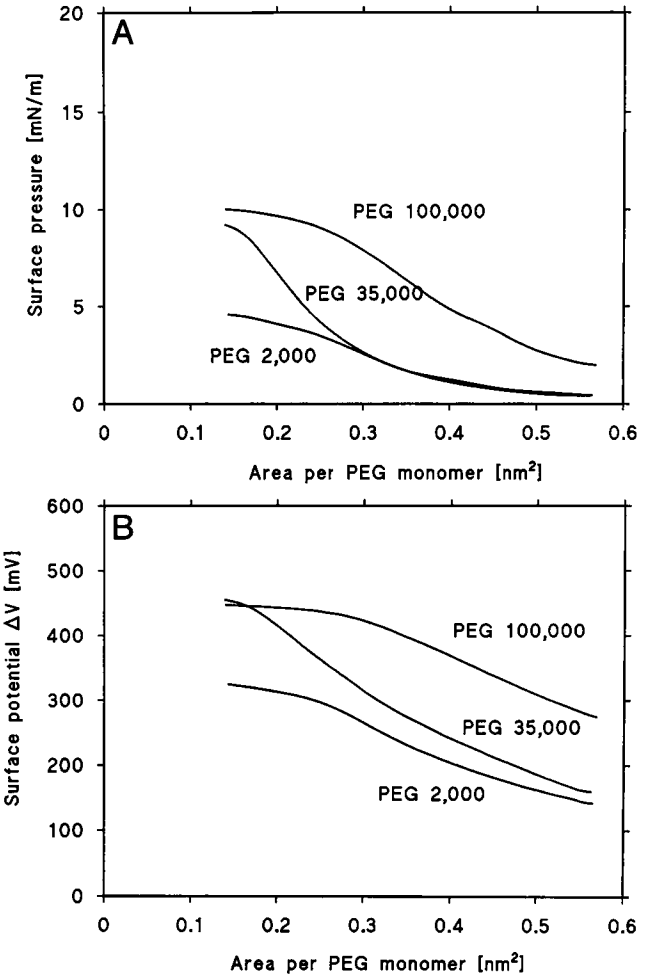

FIGURE 2 The surface properties of selected molecular weight PEGs (2000, 35,000, 100,000) spread on the air-water interface. The change in the surface potential is referenced to the air-water interface. The subphase initially contained 800 ml of 0.15 M NaCl, 2 mmol of MES, pH 6.0, T = 20°C. (*A*) Surface pressure versus area per PEG monomer. (*B*) Surface potential versus area per PEG monomer.

Effect of PEG on lipid monolayer properties

We also studied the effects of PEG on lipid monolayers of different composition and surface concentrations by either adding the polymer to the subphase or to the air-water interface.

Fig. 3 *A* illustrates the effect of adding PEG 35,000 to the subphase in the presence and absence of a DPhPC monolayer. The filled squares represent the surface potential caused by PEG 35,000 alone. In this case, the potential follows the same trend illustrated in Fig. 1. Specifically, 10^{-3}% (w/v) PEG 35,000 increases the surface potential to a plateau value ($\Delta V \sim 450$ mV) and polymer concentrations $>10^{-1}$% (w/v) cause the potential to decrease. Adding a small amount of DPhPC to the surface of a buffer solution that contains no PEG initially increases the potential to $\Delta V \sim 250$ mV ($P \sim 0$ mN/m; Fig. 3 *A*, diamonds). Increasing the polymer concentration beyond 10^{-5}% (w/v) results in potentials that are virtually identical to those caused by the polymer in the absence of lipid. The filled triangles illustrate the results of the same experiment with a densely packed DPhPC monolayer near its collapse pressure ($P \sim 46$ mN/m). In the absence of PEG, the surface potential increases to $\Delta V \sim 430$ mV which, coincidentally, is nearly equal to the

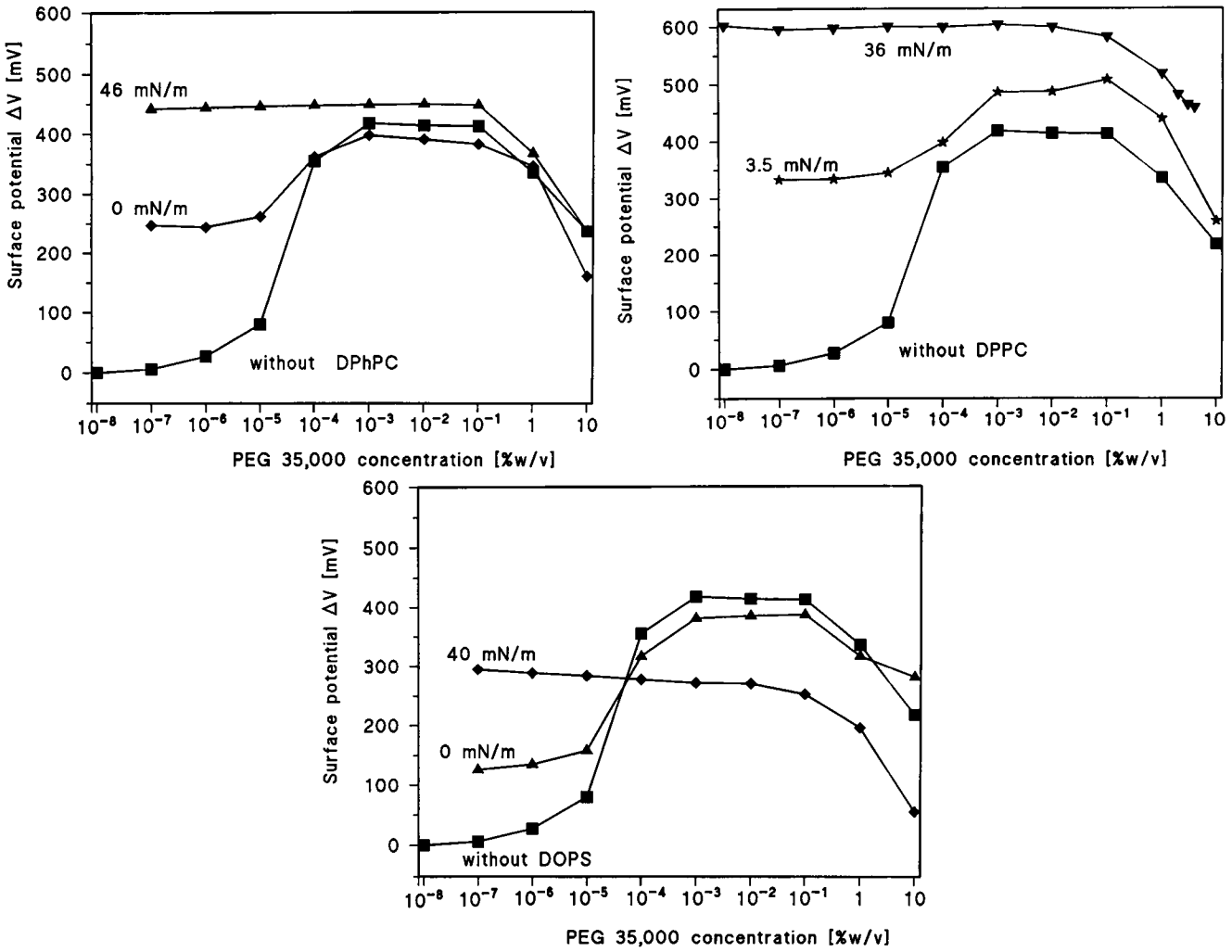

FIGURE 3 The effect of different concentrations of PEG 35,000 added to the subphase on the surface potential of lipid monolayers of different composition. The data points represent the mean values of at least three different measurements; the SD was less ±15 mV in each case. The subphase initially contained 0.15 M NaCl, 2 mmol of MES, pH 6.0, T = 20°C. The squares represent the surface potential caused by the PEG 35,000 alone. (*A*) DPhPC monolayers: the surface pressure was slightly above 0 mN/m (♦) or 46 mN/m (▲). (*B*) DPPC monolayers: the surface pressure was 3.5 mN/m (★) or 36 mN/m (▼). (*C*) DOPS monolayers: the surface pressure was negligible (▲) or 40 mN/m (♦).

maximum potential caused by PEG alone. Interestingly, the surface potential decreases when the polymer concentration exceeds 10^{-1}% (w/v). The same surface potentials result by adding PEG to the subphase first and then spreading the lipid monolayer (data not shown).

We also examined the effects of PEG on DPPC monolayers, which exhibit a phase transition between the liquid-expanded and liquid-condensed phase (monolayers spread from DPhPC show no phase transition throughout their surface-pressure isotherms (Bürner et al., 1994)). As was shown in Fig. 3 *A*, the filled squares in Fig. 3 *B* represent the potential due to PEG 35,000 in the subphase. The variation of the surface potential of low density ($P \sim 3.5$ mN/m; Fig. 2 *B*, stars) and high density ($P \sim 46$ mN/m) DPPC monolayers with PEG 35,000 concentration show the same qualitative pattern as their respective density monolayers formed by DPhPC shown in Fig. 3 *A*. Despite the fact that in the absence of polymer DPPC monolayers have a significantly greater surface potential ($\Delta V \sim 600$ mV) near their collapse pressure compared with those formed from DPhPC ($\Delta V \sim 450$ mV), the effect of PEG 35,000 on the surface potentials of DPPC and DPhPC monolayers is virtually the same.

Similarly, we studied the effect of PEG on monolayers formed by the negatively charged lipid DOPS. Near its collapse pressure, a DOPS monolayer has a lower surface potential than the maximum change in the surface potential caused by PEG. Fig. 3 *C* shows that spreading a low density DOPS monolayer increases the surface potential by $\Delta V \sim 120$ mV ($P \sim 0$ mN/m; Fig. 3 *C*, *triangles*) and that PEG 35,000 concentrations $>10^{-5}$% (w/v) cause surface potentials that are the same as those observed in the absence of lipid (Fig. 3 *C*, *filled squares*). For a dense DOPS monolayer ($P \sim 40$ mN/m; Fig. 3 *C*, *diamonds*) the change in the surface potential, which initially is $\Delta V \sim 300$ mV, is vir-

tually independent of the concentration of PEG in the subphase. However, in all cases PEG 35,000 concentrations >0.1% (w/v) cause the potential to decrease. Here the potential decreases by an amount that is virtually identical to the drop in the potential caused by PEG 35,000 alone (Fig. 3 *C, filled squares*) or that induced by the polymer in the presence of the other lipids used in this study at low or high monolayer surface pressures (Fig. 3 *A–C*).

We also measured the surface pressure and surface potential of DPhPC and DPPC monolayers in the Langmuir trough with a movable barrier. However, for these experiments, we carefully added the PEG to the surface only (i.e., not to the subphase).

Fig. 4, *A* and *B* illustrate the *P-A* and ΔV-*A* isotherms, respectively, of DPhPC monolayers in the presence or absence of PEG 35,000. Curves 1 and 2 in Fig. 4 *A* show the *P-A* relationship for monolayers spread from PEG 35,000 in the absence of lipid or of DPhPC in the absence of PEG, respectively. The area per molecule for curve 1 (PEG film only) corresponds to the nominal area per PEG monomer, whereas for curves 2–5 it represents the nominal area per lipid. Curve 2 shows that the surface pressure of a pure DPhPC monolayer starts to increase at $A \sim 1.2$ nm^2/lipid and the collapse pressure, $P \sim 48$ mN/m, occurs at ~0.68 nm^2/lipid (Bürner et al., 1994). Curves 3–5 illustrate the *P-A* isotherms that result by adding 5, 10, or 20 μg of PEG 35,000 onto the surface with an initial area 720 cm^2 (i.e., before compression). For surface pressures <10 mN/m and for low surface concentrations of PEG 35,000, the mixed monolayer *P-A* isotherms (curves 3 and 4) tend to parallel the isotherm for the polymer alone (curve 1). Increasing further the amount of PEG 35,000 on the surface (curve 5) causes the surface pressure to remain constant up to the collapse pressure for PEG 35,000 ($P \sim 10$ mN/m) for areas >1 nm^2/lipid. Compressing any of the mixed monolayers (curves 3–5) past $A \sim 1$ nm^2/lipid further results in *P-A* isotherms that are virtually identical to that caused by the lipid in the absence of the polymer (curve 2).

The surface activity of PEG and DPhPC monolayers is more evident in their surface potential isotherms, which are illustrated in Fig. 4 *B*. As in Fig. 4 *A*, curve 1 represents the surface potential of PEG 35,000 without lipid and curve 2 is the ΔV-*A* isotherm for DPhPC in the absence of polymer. Three different regimes are visible: a gas phase in which the surface potential is independent of the area per lipid ($A >$ 1.4 nm^2/lipid), a liquid-expanded phase ($A <$ 1.1 nm^2/lipid), and a steep increase that occurs between these two phases. Adding lipid and 5 μg of PEG 35,000 to a 720-cm^2 surface area before compression results in the ΔV-*A* isotherm shown in curve 3. At low nominal lipid density, this isotherm can be roughly described by superposing the isotherms for lipid (curve 2) and for PEG only (curve 1). Adding greater amounts (10 and 20 μg) of PEG 35,000 together with the lipid (curves 4 and 5) leads to yet higher surface potentials at low nominal lipid densities and causes the potential to be relatively independent of the area per lipid for $A > 0.9$ nm^2/lipid (curves 4 and 5). At higher nominal lipid densities

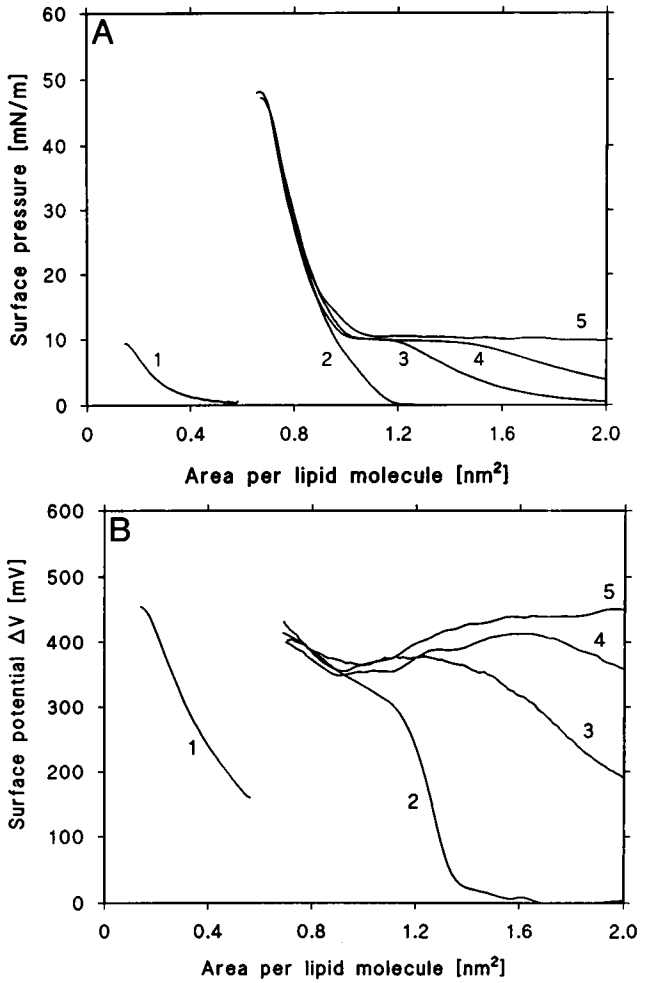

FIGURE 4 The surface properties of a monolayer in which lipid (DPhPC) and PEG 35,000 are spread simultaneously on the air-water interface. Curves 1 (in *A* and *B*) represent the surface pressure and surface potential of a PEG 35,000 monolayer alone. The *x* axis for curve 1 (PEG alone) corresponds to the area per PEG monomer. Curves 2 represent the isotherms caused by a DPhPC monolayer in the absence of polymer. For curves 3, 4, and 5, 5, 10, and 20 μg of PEG 35,000, respectively, was spread onto the 720-cm^2 surface before compression (see Materials and Methods). For curves 2–5, the *x* axis refers to the nominal area per lipid molecule. The aqueous subphases contained 0.15 M NaCl, pH 5.6. (*A*) Surface pressure versus area per lipid molecule isotherms. (*B*) Surface potential versus area per lipid molecule isotherms. The surface potentials are referred to their values in the absence of lipid and PEG, i.e., to the initial potential of the air-buffer interface.

($A < 0.9$ nm^2/lipid), the isotherms asymptotically approach the potentials observed for DPhPC monolayers in the absence of polymer (curve 2).

Fig. 5, *A* and *B* show the *P-A* and ΔV-*A* isotherms of DPPC monolayers in the presence and absence of PEG 35,000. In both figures, curve 1 corresponds to the isotherms for PEG 35,000 in the absence of the lipid. In Fig. 5 *A*, compressing a DPPC monolayer in the absence of polymer (curve 2) shows that three phases can be distinguished: the gas phase ($A > 1.2$ nm^2/lipid), the liquid-expanded phase ($0.8 < A < 1.2$ nm^2/lipid), and the liquid-condensed

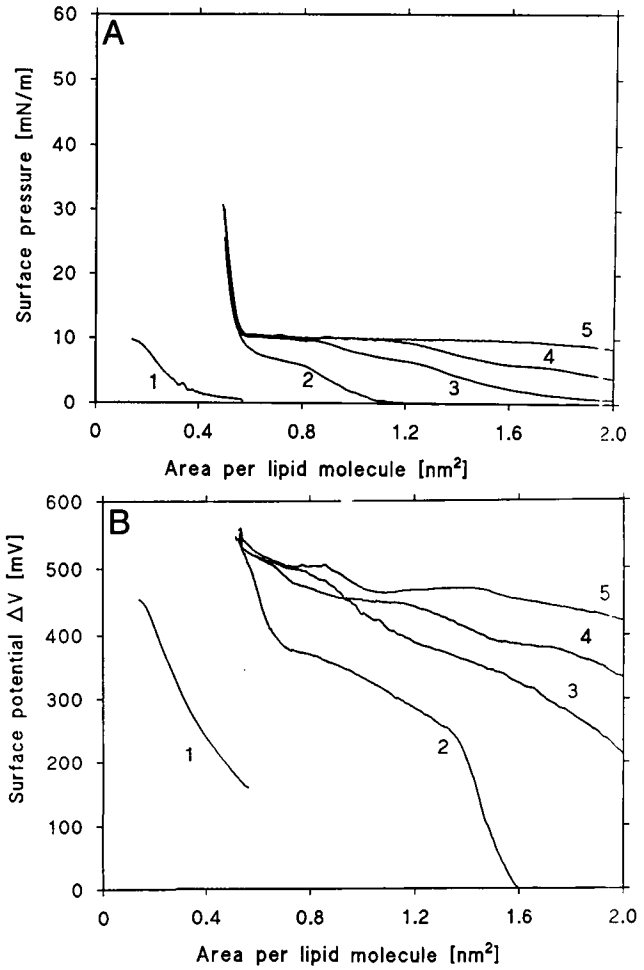

FIGURE 5 The surface properties of a monolayer where the lipid (DPPC) and PEG 35,000 are spread simultaneously on the air-water interface. Curve 1 represents that of a PEG 35,000 monolayer alone. Note that the x axis for curve 1 (PEG alone) corresponds to the area per PEG monomer. Curve 2 represents that of DPPC alone. For curves 3–5, 5, 10, and 20 μg of PEG 35,000, respectively, was spread onto the 720-cm^2 surface before compression. For curves 2–5, the x axis refers to the nominal area per lipid molecule. The aqueous subphases contained 0.15 M NaCl, pH 5.6. (*A*) Surface pressure versus area per lipid molecule isotherms. (*B*) Surface potential versus area per lipid molecule isotherms. The surface potentials are referenced to their values in the absence of lipid and PEG, i.e., to the initial potential of the air-buffer interface.

phase ($A < 0.6$ nm^2/lipid). Between the latter two regions, a phase transition occurs and the surface pressure is roughly independent of the area per DPPC molecule. Curves 3–5 correspond to the DPPC *P-A* isotherms that occur after adding 5, 10, and 20 μg, respectively, of PEG 35,000 to the surface. Note that the phase transition of DPPC is still visible even after adding sufficient PEG to alter the *P-A* isotherm (compare curves 2 and 3). The highest concentration of polymer added to the surface in the presence of DPPC increases the surface pressure to the collapse pressure of PEG 35,000 ($P \sim 10$ mN/m) (curve 5) for all nominal DPPC areas $A > 0.6$ nm^2/lipid. For all PEG concentrations used here, the *P-A* isotherms of DPPC monolayers approached that caused by DPPC alone (curve 2) by compressing the mixed monolayer to $A < 0.6$ nm^2/lipid.

The corresponding surface potential versus area isotherms for DPPC are shown in Fig. 5 *B*. Note that in the absence of the polymer the evidence for a phase transition is readily apparent (curve 2). Curves 3–5 illustrate the effect of adding 5, 10, and 20 μg of PEG, respectively, to the surface containing DPPC before compressing the mixed monolayer. Even at low nominal area per lipid molecule ($0.6 < A < 0.8$ nm^2/lipid), the effect of PEG on the surface potential is striking (Fig. 5 *B* compare curve 2 with curves 3–5). This is not the case for DPhPC (compare curve 2 in Fig. 4 *B* with curves 3–5 in that figure). This difference may occur because the surface pressure with a DPPC monolayer does not start to rise as steeply, and hence eject PEG from the surface, until the nominal area per DPPC lipid is A $\sim$ 0.6 nm^2/lipid (curves 2–5, Fig. 5 *A*), compared to A $\sim$ 1 nm^2/lipid for DPhPC.

DISCUSSION

Fig. 1 confirms that PEG added to the subphase is surface-active and can cause a substantial surface potential at the air-water interface. The maximum value of the potential induced by PEG is independent of the polymer weight (Fig. 1). This suggests that the polymer's effect on the potential is caused by the individual monomer subunits. In contrast, the concentration of PEG that is required to reach the surface potential's plateau value, and for the higher MW PEGs to initiate a subsequent decrease in the surface potential, is a function of the polymer molecular weight. Specifically, the maximum increase in the surface potential requires higher concentrations of the lower molecular weight polymers (i.e., PEGs with MW $<$ 1000). This might be caused by the presence of (unknown) endgroups on PEG. The region in which the PEG-induced surface potential was independent of the bulk concentration was observed earlier (Glass, 1968; Shuler and Zisman, 1970; Sauer et al., 1987; Kawaguchi et al., 1988; Kuzmenka and Granick, 1988; Van Oss, 1990, Sauer and Dee, 1994).

We were surprised by the decrease in the surface potential that occurs at high concentrations of PEG in the subphase. This decrease is not due to the loss of PEG from the surface, because a concomitant decrease in the surface pressure was not observed (data not shown). Also, it is not caused by an irreversible change that might occur as a result of polymer overlap or entanglement at high concentrations (the concentration at which this decrease in the surface potential occurs corresponds roughly to that at which the polymers start to overlap in the bulk aqueous phase). For example, the change in the surface potential in this regime is realized whether small aliquots of concentrated stock PEG is added to the subphase in stages (Fig. 1) or whether a highly concentrated PEG solution in the trough is diluted with PEG-free buffer (see Results). The decrease in the surface potential that accompanies higher bulk concentra-

tions of high molecular weight PEGs might be caused by a conformational change in the polymer at the surface that leads to a smaller degree of the orientation of dipoles perpendicular to the interface. Because the same effect is observed in the presence of close-packed lipid monolayers of different composition (Figs. 3 *A–C*), from which the polymer is likely to be excluded, the decrease in the potential might be due to a conformational change of the PEG that is directly adjacent to the interface.

It is unlikely that the PEG-induced changes in the surface potential are caused by impurities. First, we used PEGs from different suppliers and over a wide range of molecular weights. Second, we purified one of the PEGs using the procedure of Honda et al. (1981) and saw no difference in the ability of that polymer to increase the surface potential when added either to the subphase or as a monolayer at the air-water interface. Further arguments against impurity effects can be obtained from scaling properties. In agreement with previous studies, we found that PEG at low or intermediate bulk concentrations (up to 0.1% w/v) is characterized by a maximum in the surface potential that is independent of the molecular weight. However, the polymer concentration required to achieve this plateau value depends on the polymer molecular weight. If the surface potential was caused by impurities, then it would depend only on the amount of PEG added to the solution, not on the degree of polymerization. Similar arguments can be made for the decrease of the surface potential at high PEG concentration. Also, PEG added to the subphase caused the potential of either the air-water interface and that of three different types of lipid monolayers to change in a similar manner (Fig. 3 *A–C*). If the effects were caused by an impurity, it would need to have an identical affinity for these different interfaces.

Fig. 2 *A* confirms that monolayers formed by high molecular weight PEGs are stable and that the collapse pressure increases slightly with increasing molecular weight. The observed collapse pressures are in good agreement with those measured by others for PEGs ranging from MW 1000 to 996,000. The surface pressure caused by adding PEG to the subphase (Glass, 1968; Sauer et al., 1989; Jiang and Chiew, 1994) is similar to that measured for PEG spread at the air-water interface (Shuler and Zisman, 1970; Nitsch et al., 1987; Sauer et al., 1987; Kuzmenka and Granick, 1988; Kawaguchi and Nishida, 1989; Sauer et al., 1989). In agreement with earlier measurements on monolayers spread from PEG 90,000 (Shuler and Zisman, 1970), compressing the polymer films resulted in a maximum surface potential of ΔV = 440 mV, which is independent of the polymer molecular weight. We also found that the surface potential that occurs by compressing a PEG film spread at the air-water interface ($P \sim 10$ mN/m, Fig. 2 *B*) is virtually identical to the maximum value of the potential caused by adding PEG to the subphase (ΔV = 440 mV, Fig. 1).

Fig. 3 *A–C* show that moderate concentrations of PEG in the subphase increase the surface potential of low density monolayers spread from DPhPC, DPPC, and DOPS. This effect could be explained either by an orientation of the lipid caused by PEG or by the incorporation of oriented PEG into the air-lipid-water interface. The latter possibility is supported further by our observation of an increase in the surface pressure after injecting PEG into the subphase (data not shown). Fig. 3 *A–C* also show that high concentrations of polymer (>0.1% w/v) decrease the surface potentials of lipid monolayers by virtually the same amount even when the lipids are densely packed. This is especially interesting because the three lipids we used have markedly different surface potentials at their collapse pressures in absence of PEG ($\Delta V \sim$ 430, 600, and 350 mV for DPhPC, DPPC, and DOPS, respectively). It is noteworthy that this effect is also observed in the absence of lipids and is likely to be entirely due to some property of the polymer. PEG is normally a random coil in aqueous solution. For polymer concentrations greater than a molecular weight-dependent value, the random coils start to overlap and a higher ordered conformation of PEG might form. The latter conformation might adsorb readily to the air-water or lipid-water interface and reduce the surface potential.

Some of our results are in qualitative agreement with an earlier study of the action of PEG solutions on the surface pressure and surface potential of lipid monolayers (Maggio et al., 1976). However, in those experiments the surface potential of the PEG-water solution was taken as the reference. Thus, whenever the surface potentials of the lipid and the PEG change by the same amount (with the same slope), the lipid monolayer's surface potential would remain unchanged. It is for this reason that Maggio and co-workers did not observe a decrease in the surface potentials at high polymer concentrations that we report here.

CONCLUSION

PEGs are decisively surface-active and have a bimodal effect on the surface potentials of air-water interfaces and lipid monolayers. Are PEG's surface-active properties reported here of relevance to bilayer and cellular studies? There is no general agreement on the value of the surface pressure of solvent-free (or solvent-containing) planar bilayer membranes or of lipid vesicles (see the discussion of this point in Rebecchi et al., 1993). Some recent studies suggest that the surface pressure of bilayers is ~30 mN/m (Blume, 1979; Seelig and MacDonald, 1989; but see MacDonald and Simon, 1987). Such high surface pressures certainly exclude PEG from the surface when the polymer is spread at the air-lipid-water interface (Figs. 4 *A* through 5 *B*). However, when PEG is added to the bulk aqueous phase at sufficiently high concentrations, it alters the surface potentials of three different types of lipid monolayers, even when the lipids are densely packed (Fig. 3 *A–C*). Thus, if there is a correspondence between the states of lipids in monolayers and bilayers, PEG should influence the potential at the bilayer-water interface in a polymer concentra-

tion- and PEG molecular weight-dependent manner. It is interesting to note that the polymer concentrations that cause this effect are in the ranges of those used in the study of PEG-induced fusion of liposomes and cells and in the biophysical characterization of ion channels reconstituted into planar bilayer membranes.

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