The Structure and Stability of Phospholipid Bilayers by Atomic Force Microscopy

S. W. Hui,* R. Viswanathan,‡ J. A. Zasadzinski,‡ and J. N. Israelachvili‡

* Department of Biophysics, Roswell Park Cancer Institute, Buffalo, NY 14263, and ‡ Department of Chemical and Nuclear Engineering, University of California, Santa Barbara, CA 93106 USA

ABSTRACT   Atomic force microscopy (AFM) was used to investigate the structure, stability, and defects of the hydrophilic surfaces of Langmuir-Blodgett bilayer films of distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylethanolamine (DPPE) in the solid phase, and dilinoleoylphosphatidylethanolamine (DLPE) in the fluid phase. Their relative resilience to external mechanical stress by the scanning tip and by fluid exchange were also investigated. DPPE monolayers showed parallel ridges at the surface with a period of 0.49 nm, corresponding to the rows of aligned headgroups consistent with the known crystallographic structure. DSPC and DLPE monolayers did not show any periodic order. The solid DSPC and DPPE monolayers were stable to continued rastering by the AFM tip; however, the stability of DLPE monolayers depended on the pH of the aqueous environment. Structural defects in the form of monolayer gaps and holes were observed after fluid exchange, but the defects in DLPE monolayer at pH 11 were stable during consecutive scanning. At pH 9 and below, the defects induced by fluid exchange over DLPE monolayers were more extensive and were deformed easily by consecutive scanning of the AFM tip at a force of 10 nN. The pH dependence of resilience was explained by the increasing bending energy or frustration due to the high spontaneous curvature of DLPE monolayers at low pH. The tangential stress exerted by the AFM tip on the deformable monolayers eventually produced a ripple pattern, which could be described as a periodic buckling known as Shallamach waves.

INTRODUCTION

Phospholipid bilayers form the basic framework of biological membranes. Far from being merely a passive structural matrix, membrane phospholipids play an active role in signal transduction (Berridge and Irvine, 1984) and functional modulation, some of which are controlled by the physical state of the membrane phospholipids (Cullis et al., 1985). For instance, phospholipid molecules with unequal cross-sections of the headgroups and hydrocarbon tails tend to form spontaneously curved monolayers at a polar/nonpolar interface (Israelachvili et al., 1976). In bilayers of these phospholipids, both monolayer halves are constrained to a common curvature and pay a price in bending energy. This bending or frustration energy contributes to the instability of the bilayer, which is then liable to transform to a non-bilayer structure when sufficient energy is provided to overcome the polymorphic transition energy barrier (Seddon, 1990). This stored energy in a bilayer is believed to modulate the functions of many membrane proteins, especially in energy-transducing membranes (Hui and Sen, 1989).

The stability of phospholipid monolayers and multilayers on substrates is a major concern for the use of such layers in electronic, nonlinear optics and biocompatible materials (Roberts, 1990). The stability of phospholipid monolayers constrained to a planar substrate is of particular importance to the application of phospholipid monolayers and multi-

layers as matrices for enzymes in biosensors. Monolayers or multilayers (such as Langmuir-Blodgett films) of ordered molecules in a well-defined crystallographic lattice are significantly more stable than disordered films (Schwartz et al., 1992; Zasadzindki, 1994). Increasing the number of layers also contributes to the stability of the film, most likely due to strong interlayer interactions (Schwartz et al., 1992; Zasadzindki, 1994). It is postulated that phospholipid monolayers with high spontaneous curvatures, when constrained as monolayers or multilayers on a planar substrate, would be unstable due to the stored bending energy (Hui and Sen, 1989). Hence, we expect to see a significant difference in the properties of saturated versus unsaturated phospholipids and ethanolamine versus choline headgroups.

Phosphatidylethanolamine (PE) is a common phospholipid in biological membranes and is usually abundant in highly active membranes. PE with unsaturated acyl chains is not stable in the bilayer form and tends at equilibrium to convert to high curvature structures, such as inverted cubic or inverted hexagonal phases, at room temperature and at neutral pH. At pH > 9, the PE headgroup is charged, and because of electrostatic repulsion, the equivalent headgroup cross-sectional area increases to be commensurate with that of the tails. Therefore PE is more stable in the bilayer form at high pH. The pH-dependent stability of PE bilayers is well documented (Sen et al., 1991). We report here our study of the stability of both symmetric and asymmetric PE bilayers deposited on mica substrates by atomic force microscopy (AFM). AFM enables us to study the packing order of headgroups of saturated or unsaturated PE at molecular resolution under ambient conditions and full hydration, and to monitor the formation of defects of unsaturated PE as a function of pH of the buffer solution and the applied force of the AFM tip.
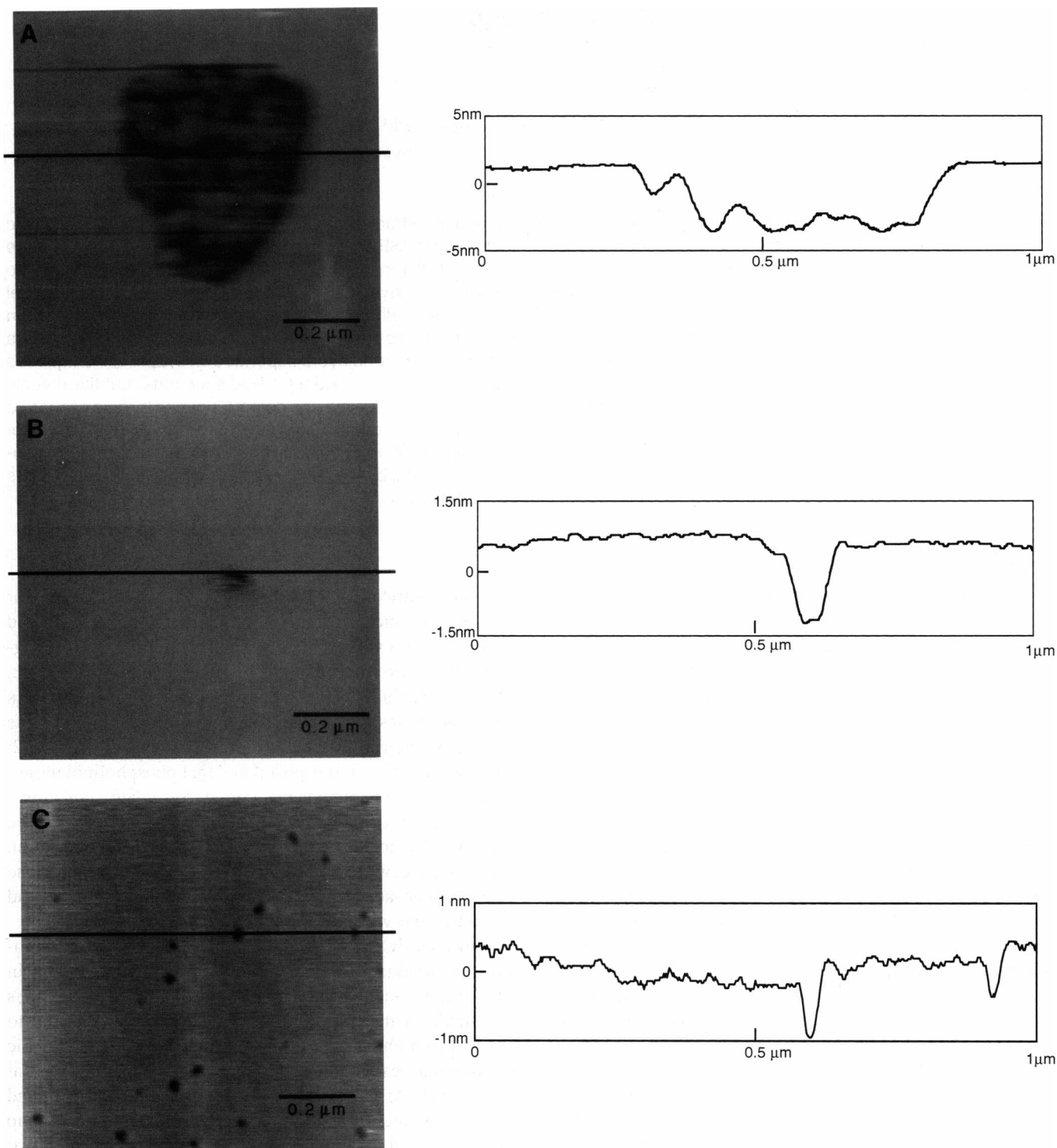
FIGURE 1 Wide scan area AFM micrographs of hydrophilic side of (A) a DPPE/DPPE bilayer; (B) a DPPE/DSPC bilayer; (C) a DPPE/DLPE bilayer imaged under water. Z-direction profiles indicate depths of holes of the deposit film.

MATERIALS AND METHODS

Dipalmitoylphosphatidylethanolamine (DPPE), dilinoleoylphosphatidylethanolamine (DLPE), and distearoylphosphatidylcholine (DSPC) were purchased from Avanti Polar Lipids (Alabaster, AL). All lipids were dissolved in chloroform at 2 mg/ml. Monolayers of these lipids were spread on an aqueous (water from a Milli-Q (Bedford, MA) system was used) subphase in a commercial NIMA (Warwick Science Park, Coventry, CV4

7EZ, UK) trough. After allowing time for solvent evaporation, the monolayer was compressed at 12 cm^2/min, and the pressure-area (p-A) curve was recorded.

The mica substrates were cleaned by repeated rinsing in ethanol and Millipore water and cleaved in a dust-free hood just before submersion in the subphase of the trough, before the monolayer was spread. DPPE was chosen to be the common first layer because of its stability on mica substrates. This first monolayer was transferred at a surface pressure of 40

mN/m by raising the mica substrate vertically through the air-water interface at 3 mm/min. The remaining DPPE monolayer at the trough surface was then removed, and the trough was cleaned before spreading the second monolayer of the desired phospholipid. The second monolayer was deposited by passing the DPPE-coated substrate vertically into the trough. The mica substrate now carried the first monolayer of DPPE, with headgroups facing the hydrophilic mica surface, and the second monolayer of the phospholipid to be imaged, with headgroups facing the aqueous subphase. The transfer ratio was usually near unity for DPPE and DSPC, and slightly lower for the unsaturated PEs.

The bilayer-coated mica was kept under water during transfer (no more than 30 min, unless specified, elapsed from deposition to imaging) to the liquid cell of a Nanoscope II or III AFM (Digital Instruments, Inc., Santa Barbara, CA). In some experiments, the water in the liquid cell was replaced by buffered solutions of a given pH. AFM images were obtained under ambient conditions using a silicon nitride tip on a cantilever with a spring constant of 0.12 N/m using standard techniques (Schwartz et al., 1993). The specimens were initially scanned at 3 μm × 3 μm for gross features. The best molecular resolution was achieved in the "force mode," that is, scanning the tip at constant height and measuring spring deflection. Typical forces were 10^{-8} N.

RESULTS

Effect of headgroups

The AFM images only the hydrophilic surface of the second, or top, monolayer of the bilayer. Hence, the structures of the second or top monolayers of DSPC and DPPE can be directly compared. DPPE is used as the first layer on mica, whereas either DPPE or DSPC is used to form the second or surface monolayer that was imaged. These specimens are denoted as DPPE/DPPE or DPPE/DSPC, respectively. The pressure-area characteristics of these lipids are recorded before transfer to the substrates and were consistent with those in the literature (McConnell, 1990; Mohwald, 1991). The shapes of the π-A curves of DSPC and DPPE are very similar at 22°C, except that DPPE has a smaller area per molecule at a given surface pressure. Both DSPC and DPPE monolayers are at the solid or S-phase at the transfer pressure of 40 mN/m; DPPE occupies about 38 Å^2 per molecule while DSPC occupies 42 Å^2 per molecule.

Low magnification (1 × 1 μm scan) AFM micrographs taken under water or buffer showed that the DPPE and DSPC surface layers were quite flat and featureless, except for occasional holes or empty patches. The holes in DPPE/DPPE bilayers are 5.4 ± 0.1 nm deep, indicating both the first (bottom) and the second (top) monolayers were missing in these holes (Fig. 1 A). This confirmed that the surface being imaged was the hydrophilic or headgroup surface under water, in agreement with previous AFM studies of Langmuir-Blodgett phospholipid bilayers (Egger et al., 1990; Weisenhorn et al., 1990, 1991; Zasadzinski, 1991). However, in AFM studies of
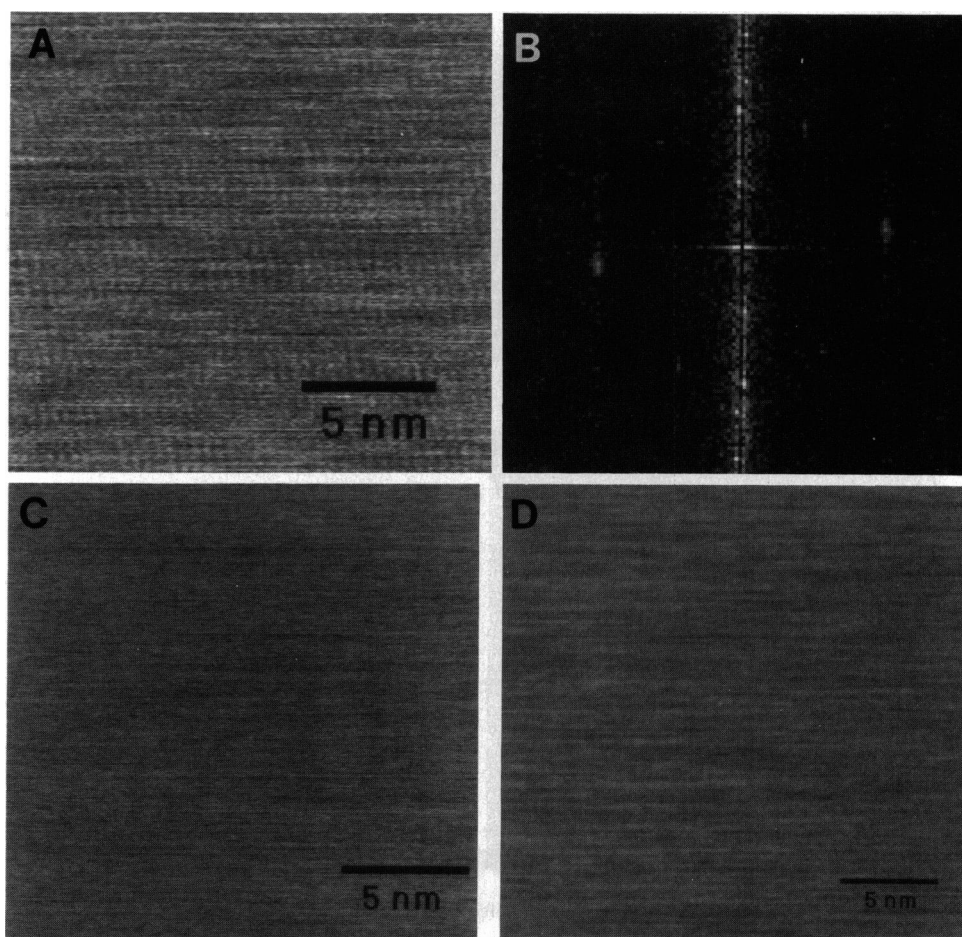

FIGURE 2 High resolution AFM image of (A) a DPPE/DPPE bilayer showing ridges (running top to bottom in the image) with a periodicity of 0.49 nm. (B) Two-dimensional Fourier transform of the micrograph A showing well-defined crystallographic lattice structure with a roughly hexagonal symmetry of spacing of 0.5 nm. (C) DPPE/DSPC bilayer; (D) DPPE/DLPE bilayer. Neither the DPPE/DSPC or DPPE/DLPE surface shows any evidence of a lattice structure.

cadmium arachidate films under water, the hydrophilic surface was unstable and the tip burrowed through to the hydrophobic or tailgroup surface underneath (Schwartz et al., 1992; Zasadzinski et al., 1994). This suggests that the coupling between monolayers in phospholipids is significantly different than in fatty acid salts. The DPPE/DSPC bilayers had only few holes, which were 2.1 ± 0.1 nm deep, indicating only the top layer is missing (Fig. 1 B).

Higher magnification (20 × 20 nm scan) AFM images of DPPE/DPPE showed well defined, regularly spaced, parallel ridges about 0.49 nm apart. The direction of the ridges varied from area to area and was independent of the AFM scanning direction (Fig. 2 A). Two-dimensional Fourier transforms of all high magnification micrographs gave at least a pair of strong diffraction spots at 0.49 ± 0.01 nm spacing, corresponding to the separation between the rows of molecules visible in Fig. 2 A. Fourier analyses of several micrographs show four additional weaker spots, with roughly hexagonal symmetry, and also at roughly 0.5 nm spacing, but the absolute location of these spots was not consistent from micrograph to micrograph. One such example is given in Fig.

2 B. Similar scans of DPPE/DSPC bilayers failed to reveal any ordered patterns (Fig. 2 C). Due to problems with the stability of the sample in the liquid cell, the amount of time we could image the sample was limited, and this limited the resolution of the image and the Fourier transform (Schwartz et al., 1993).

Effect of chain order

The effects of lipid saturation and chain order on the stability and structure of bilayers was investigated by comparing DPPE (saturated) and DLPE (unsaturated) monolayers. The π-A curve of DLPE is quite different than those of DPPE and DSPC in that there is no detectable phase transition of the monolayer at the air-water interface. The monolayer has nearly constant compressibility starting at almost zero surface pressure. This indicates that the liquid-solid transition temperature of DLPE is well below the experimental temperature of 22°C (McConnell, 1990; Möhwald, 1991). The transfer of the second monolayer of DLPE on DPPE is made at an arbitrary pressure of 30 mN/m, to be well away from
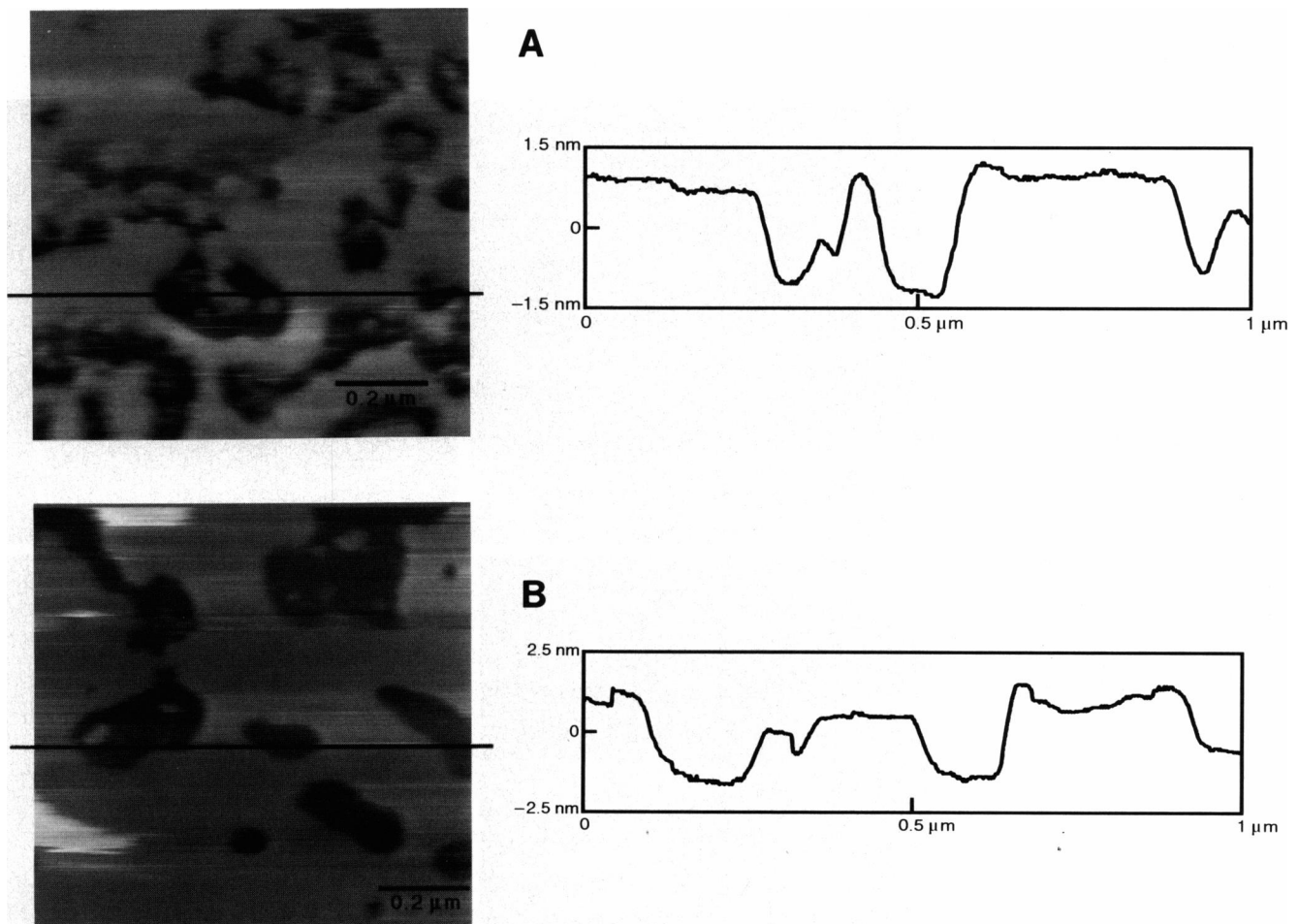

FIGURE 3   Height profile of DPPE/DLPE bilayer defects induced by changing the buffer solutions to a final pH value of pH = 5 (A) and pH = 11 (B) as recorded at the initial AFM scan.
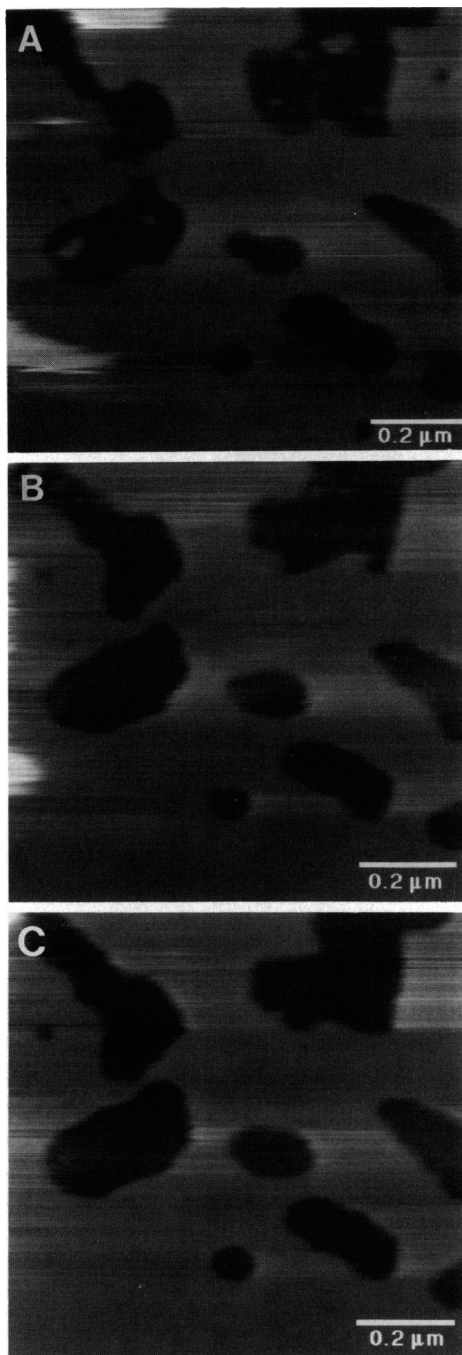
FIGURE 4 Series of consecutive AFM scans of DPPE/DLPE bilayer at pH = 11. The monolayer-deep holes in the surface are stable to repeated AFM scanning at pH 11, although there is some loss of the small islands within the larger holes.

the usual collapse pressure of about 45 mN/m (McConnell, 1990; Möhwald, 1991).

At low magnification (1 × 1 μm scans), the surface of DPPE/DLPE at room temperature is featureless apart from a few small depressions (Fig. 1 C). These depressions in the bilayer are less than 1 nm deep and generally <50 nm in diameter. Most likely, these are not holes in the monolayer or bilayer, as observed in Fig. 1, A and B, but rather local areas of lower phospholipid density, and consequently, areas of higher compressibility. Occasionally small lumps of materials are seen scattered on the surface. No ordered patterns are observed at high magnification (Fig. 2 D), which is consistent with the liquid-like organization of the monolayer on the water surface.

Effect of pH on stability

Monolayers of unsaturated PEs, such as DLPE, have a high spontaneous curvature at and below neutral pH, so they are not expected to be stable in the lamellar phase (Hauser et al., 1981; Gruner et al., 1986; Boni and Hui, 1983). A metastable planar monolayer of DLPE may be disrupted by an external force which might not cause damage to a stable monolayer. To investigate this possibility, DPPE/DLPE bilayers were subjected to various pHs by replacing their immersion liquid after the formation of the asymmetric bilayers. The mechanical disturbance during the fluid exchange was sufficient to induce defects on the surface layers. The degree of defect formation, and, more significantly, the tolerance to repeated scanning of the AFM tip, were highly pH-dependent, as detailed below.

Some induced defects are seen at the surface of DPPE/DLPE after the water is replaced by a buffered solution at pH 11. The defects take the form of randomly scattered troughs and holes of about 100 nm wide and with a uniform depth of 2.5 ± 0.15 nm (Fig. 3). The depth corresponds to the thickness of one monolayer. Apparently only the top monolayer of the more fluid DLPE is disrupted. However, these defects are relatively stable to repeat scanning of the AFM tip. Fig. 4, A–C, shows consecutive scans of the same area of the DPPE/DLPE bilayer. Apart from the removal of some elevated spots and some fine features (islands) in the large hole, the basic defect features remain the same size and shape. This observation is in distinct contrast to that of the same bilayers exposed to buffered solutions at pH 9 and lower. Fig. 5 is a consecutive scan series of DPPE/DLPE in a solution buffered at pH 5. The initial defect pattern was significantly more extensive and more convoluted than those observed at pH 11 (Fig. 4). At this pH, the defects become wider after only one scan, and much of the surface monolayer material was removed (or brushed aside by the tip) after a few scans. Eventually nearly all the top monolayer material was removed. We then observed a pattern of roughly parallel troughs and valleys in the monolayer at a spacing of 10–100 nm. These linear features always aligned perpendicular to the scanning direction. This behavior is common to all DPPE/DLPE bilayers studied at pH 9 and below.

DISCUSSION

Phospholipid monolayer stability is an important consideration both in the understanding of functional modulation of biomembranes as well as in coating biosensors and other devices. AFM provides an important way to measure this stability and to observe the nature of the defects resulting
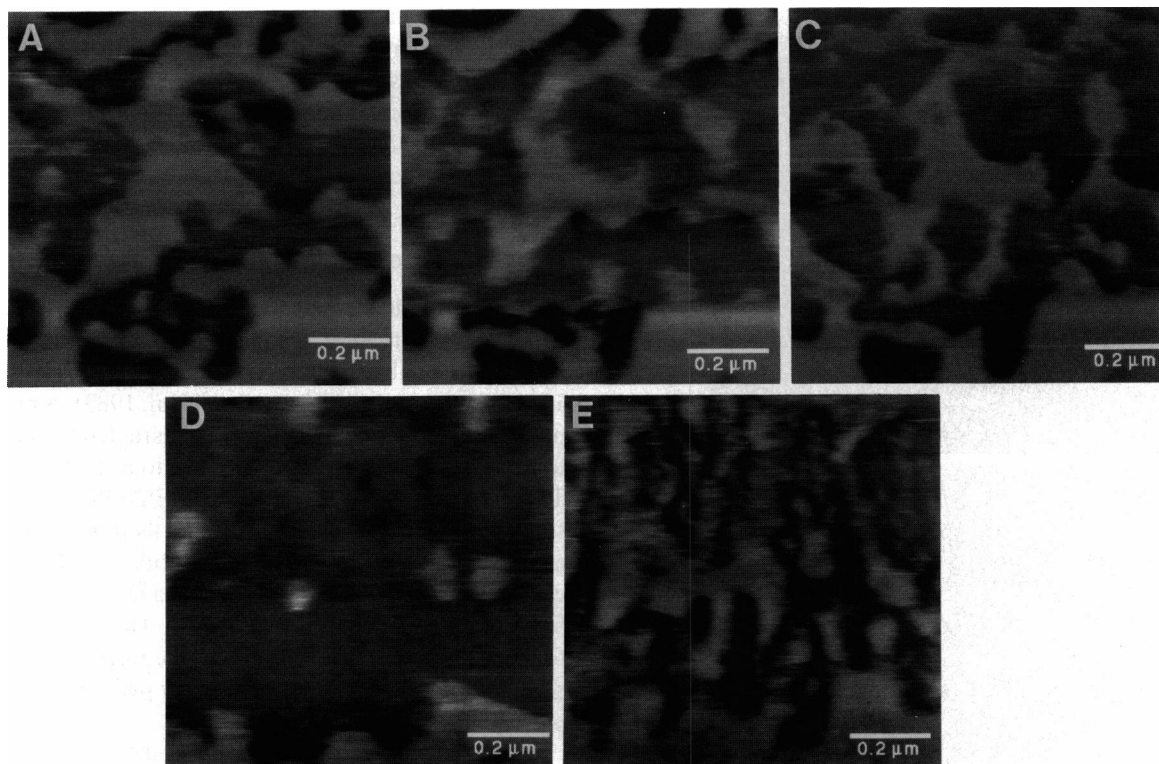
FIGURE 5   Series of consecutive AFM scans of DPPE/DLPE bilayer at pH = 5. The top monolayer of the unsaturated, fluid DLPE is sequentially removed from A to D, suggesting that the cohesion of the monolayer at pH 5 is significantly less than at pH 11. (E) After repeated imaging, there appears a somewhat regular pattern of linear features perpendicular to the scanning direction of the AFM. This phenomena is common to any soft material when rubbed by a harder material, and the ripples are known as Shallamach waves (Schallamach, 1971).

from instability. There have been several reported studies of phospholipid monolayers by AFM. The natural way to study bilayer or monolayer surface is with the phospholipid head-groups in water. Hansma and co-workers (Egger et al., 1990; Weisenhorn et al., 1990, 1991) have reported the observation of PE and PG monolayers on OTS, while Zasadzinski et al. (1991) reported on DPPE/DPPE bilayers. The first study found that PE surface consists of parallel ridges of 0.9 nm in spacing, the latter reported a 0.7–0.9 nm repeating period. No repeating order has been seen along the ridges. Although Weisenborn et al. compared their 2-dimensional surface pattern of DMPG with crystallographic data, no comparison was made for their PE data.

Our finding of parallel ridges with a periodicity of 0.49 ± 0.01 nm differs from the values of previous reports. It is distinct from the hexagonal pattern of mica with a hole-to-hole distance of 0.52 nm (Schwartz et al., 1993). The 0.49-nm rows agree well with crystallographic structure of dilauroyl PE (Elder et al., 1977; Hauser et al., 1981). The dilauroyl PE crystal structure has an alternating antisymmetric subunit repeat of the headgroups in the plane of the bilayer surface with a spacing of 9.9 Å. Each subunit has two rows of headgroups offset in the direction normal to the bilayer, producing a spacing of 4.9–5.0 Å between rows. PE has a characteristic alignment of headgroups stretching along the same direction end-to-end, and its headgroups are believed to form a hydrogen bond network (Sen et al., 1988;

Elder et al., 1977; Hauser et al., 1981). The ridges we found are most likely to be rows of aligned headgroups. The height of the ridges (about 0.4 nm) also indicate that the headgroup is aligned on the side as depicted by crystallographic data (Elder et al., 1977; Hauser et al., 1981). The headgroups in PC, as deduced from crystallographic data, are not coplanar and aligned end-to-end as in the case of PE. Hence, the surface is more uniform and as a result, more difficult for AFM imaging. So far no ordered structure has been reported from AFM imaging of PC surface. Furthermore, in the "solid" state of their bilayers, PE is more likely to be in the crystalline L_c phase, while PC is in the less ordered gel or L_β, phase. In the fluid state, even PE headgroups are not ordered, hence it is not surprising that no ordered structures are seen in the unsaturated PEs.

There are a number of factors that affect the stability of phospholipid monolayers on a solid substrate. The tightness of packing, in terms of area per molecule, the ordering of molecular packing, and the number of layer are a few known factors. One of the factors that has not been examined is the stored bending energy or frustration of a monolayer constrained to a plane when the spontaneous curvature of the monolayer is high. An external disturbance that provides sufficient energy to overcome the inter-monolayer adhesion should disrupt these layers much more readily than those without any stored frustration energy. In the absence of this external disturbance, the bilayer can remain in a metastable

form. The seemingly intact bilayers of DPPE/DLPE, kept under the same subphase liquid from the Langmuir trough, was in this metastable state, which was found to remain the same overnight in one of our experiments. However, the stresses associated with exchanging the liquid in the liquid cell of the AFM is sufficient to disrupt the DLPE monolayer, especially if the fluid is not presaturated with the same lipid. The number and extent of defects, seen as gaps and holes of the top monolayer, is expected to be dependent on both the degree of frustration as well as on the extent of external disruption. Because the latter factor during fluid exchange is not controllable, we make no effort to quantify the defects. Qualitatively, there are more defects found in the monolayers at pHs 5 and 9 than at pH 11. Assuming the degree of disturbance to be the same for all samples during the fluid exchange, the amount of disruption does increase with increasing frustration as expected. It is interesting to note that the defects are limited only to the surfaces or the top monolayers, which are metastable.

More significant is that the instability of these metastable top layers are manifested as the susceptibility to the mechanical force of the AFM tip scanning. The same force is applied to these samples so the degree of distortion reflects the mechanical stability of the top layer. Figs. 4 and 5 illustrate the general result that the top DLPE monolayer is much more stable at pH 11 than in pH 5. At pH 11, DLPE is charged and prefers the bilayer form at room temperature. The spontaneous curvature of DLPE at pH 11 at room temperature is believed to be very small and cannot be measured by the hexagonal tube size method (Gruner et al., 1986) for the same reason. At pH below 9, the hexagonal inter-tube spacing of DLPE at 20° is 6.9 nm (Boni and Hui, 1983), from which one may estimate the spontaneous curvature to be $\sim$0.4 nm^{-1}. The frustration energy may reach several kT if a bending elastic modulus of 10^{-19} J is assumed (Evans and Skalak, 1979). A much higher susceptibility to mechanical disruption is therefore expected.

The ultimate deformation of the disrupted top monolayer is also an interesting phenomenon. The deformed surface takes the form of corrugated patterns, with periodic ridges invariably perpendicular to the direction of tip scanning. These features, and similar but much larger patterns of ridges and troughs in the surface of polystyrene on mica after continuous AFM tip scanning (Leung and Goh, 1992), are nanometer scale examples of a more general phenomena known as Shallamach waves (Schallamach, 1971). Similar undulating patterns on macroscopic length scales are observed when any hard object slides over a soft, viscoelastic surface (or a soft object slides over a hard surface). A common example is the washboard effect often observed on dirt roads. The soft surface, here the monolayer, buckles when its cohesion is less than the compressive strain induced by the combined load and adhesion of the the AFM tip as it slides across the surface. After repeated exposure to the AFM tip, these buckling patterns grow into a regular series of ridges and troughs. These Shallamach waves are general to any soft surface that is deformed by the AFM tip and not a result of the particular lipid. However, the threshold strain for buckling appears to be significantly less for the unsaturated, frustrated DLPE monolayers than for the saturated, nonfrustrated DPPE or DSPC monolayers. This is the first time that this phenomenon is observed at the nanometer scale in lipid monolayers. This observation may provide a new way to measure elastic deformation by AFM and serve as a precaution to interpret surface topography revealed by scanning force microscopy.

REFERENCES

Berridge, M. J., and R. F. Irvine. 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature*. 312:315–321.

Boni, L. T., and S. W. Hui. 1983. Polymorphic phase behavior of dilinoleoyl phosphatidylethanolamine and palmitoyl-oleoyl phosphatidylcholine mixtures. Structural changes between hexagonal, cubic and bilayer phases, *Biochim. Biophys. Acta*. 731:4763–4769.

Cullis, P. R., M. J. Hope, B. de Kruijff, A. J. Verkleij, and C. P. S. Tilcock. 1985. Structural properties and functional roles of phospholipids in biological membranes. *In* Phospholipids and Cellular Regulation, Vol. 1. J. K. Kuo, editor. CRC Press, Boca Raton, FL. 1–59.

Egger, M., F. Ohnesorge, A. L. Weisenhorn, S. P. Heyn, B. Drake, C. B. Prater, S. A. C. Gould, P. K. Hansma, and H. E. Gaub. 1990. Wet lipid proteins imaged at submolecular resolution by atomic force microscopy, *J. Struct. Biol.* 103:89–94.

Elder, M., P. B. Hitchcock, R. Mason, and G. G. Shipley. 1977. A refinement analysis of the crystallography of the phospholipid, 1,2-dilauroyl-DL-phosphatidylethanolamine, and some remarks on lipid-lipid and lipid-protein interactions, *Proc. Roy. Soc. Lond.* A354:157–170.

Evans, E., and A. Skalak. 1979. The Mechanics and Thermodynamics of Biomembranes. CRC Press, Boca Raton, FL.

Gruner, S. M., V. A. Parsegian, and R. P. Rand. 1986. Directly measured deformation energy of phospholipid HII hexagonal phases. *J. Chem. Soc. Faraday Trans.* 81:29–37.

Hauser, H., I. Pascher, R. H. Pearson, and S. Sundell. 1981. Preferred conformation and molecular packing of phosphatidylethanolamine and phosphatidylcholine. *Biochim. Biophys. Acta.* 650:21–51.

Hui, S. K., and A. Sen. 1989. Effects of lipid packing on polymorphic phase behavior and membrane properties. *Proc. Natl. Acad. Sci. USA.* 86: 5825–5829.

Israelachvilli, J. N., D. J. Mitchell, and B. W. Ninham. 1976. Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers. *J. Chem. Soc. Faraday Trans.* 72:2525–2568.

Leung, O. M., and C. M. Goh. 1992. Orientational ordering of polymers by atomic force microscope tip-surface interaction. *Science.* 255:64–66.

McConnell, H. M. 1990. Structures and transitions in lipid monolayers at the air-water interface. *Annu. Rev. Phys. Chem.* 77:398–445.

Möhwald, H. 1991. Phospholipid and phosolipid-protein monolayers at the air-water interface. *Annu. Rev. Phys. Chem.* 41:441–485.

Roberts, G. G. 1990. Langmuir-Blodgett Films. Plenum Press, New York.

Schallamach, A. 1971. How does rubber slide? *Wear.* 17:301–312.

Schwartz, D. K., J. Garnaes, R. Viswanathan, S. Chiruvolu, and J. A. Zasadzinski. 1993. Quantitative lattice measurements of thin Langmuir-Blodgett films by atomic force microscopy. *Phys. Rev. E.* 47:452–460.

Schwartz, D. K., J. Garnaes, R. Viswanathan, and J. A. Zasadzinski. 1992. Surface order and stability of Langmuir-Blodgett films. *Science*. 257:508–511.

Seddon, J. M. 1990. Structure of the inverted hexagonal (HII) phase, and nonlamellar phase transitions of lipids. *Biochim. Biophys. Acta*. 1031:1–69.

Sen, A., T. V. Isac, and S. W. Hui. 1991. Bilayer packing stress and defects in mixed dilinoleoyphosphatidyl-ethanolamine and palmitoyloeoylphosphatidylcholine and their susceptibility to phospholipase A_2. *Biochemistry*. 30:4516–4521.

Sen, A., P. W. Yang, H. H. Mantsch, and S. W. Hui. 1988. Extended hydrogen bonded structures of phosphatidylethanolamine. *Chem. Phys. Lipids*. 47:109–116.

Weisenhorn, A. L., B. Drake, C. B. Prater, S. A. C. Gould, P. K. Hansma, F. Ohnesorge, M. Egger, S. P. Heyn, and H. E. Gaub. 1990. Immobilized proteins in buffer imaged at molecular resolution by atomic force microscopy. *Biophys. J.* 58:1251–1258.

Weisenhorn, A. L., M. Egger, F. Ohnesorge, S. A. C. Gould, S.-P. Heyn, H. G. Hansma, R. L. Sinsheimer, H. E. Gaub, and P. K. Hansma. 1991. Molecular-resolution images of Langmuir-Blodgett films and DNA by atomic force microscopy. *Langmuir*. 7:8–12.

Zasadzinski, J. A. N., C. A. Helm, M. L. Longo, A. L. Weisenhorn, S. A. C. Gould, and P. K. Hansma. 1991. Atomic force microscopy of hydrated phosphatidylethanolamine bilayers. *Biophys. J.* 59:755–760.

Zasadzinski, J. A., R. Viswanathan, L. Madsen, J. Garnaes, and D. K. Schwartz. 1994. Langmuir-Blodgett films. *Science*. 263:1726–1733.