Force and Adhesion Measurements between Hydrogen-Bonded Layers of Glycine-Functionalized Amphiphiles

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The structural integrity of proteins, DNA, and supramolecular assemblies[1] depends critically on the strengths of hydrogen bonding, electrostatic, van der Waals, hydrophobic, and steric forces operating within these structures. Micromechanical measurements have made possible the direct probing of these individual molecular interactions. Using the surface force appartus (SFA)[2] and the atomic force microscope (AFM), the strengths of receptor/ligand,[3] DNA base-pairing,[4] and functional group interactions[5] have been reliably quantified. Here, we employ the SFA to make a realistic, mechanical measurement of the structural integrity of model Langmuir−Blodgett (LB) layers[6] which are stabilized by a combination of intermolecular forces.

We have covalently linked the amino acid glycine to an amphiphilic molecule by a flexible synthetic technique,[7] with the N-terminus of glycine closest to the amphiphile and the C-terminus (acid form) distal from it (Figure 1).

For the SFA experiment, bilayers of dipalmitoylphosphatidyl-ethanolamine (DPPE) and the glycine amphiphile $(C_{16})_2$-Glu-C_2-Gly were compressed to the high-density regime and deposited onto mica following a standard protocol.[8] Using AFM, the bilayers were found to be largely defect-free and stable over a period of days. A simple space-filling model argues that this composite bilayer should have a thickness of about 58 Å in anhydrous form.[8] Force measurements (Figure 2) were made in Milli-Q-purified water (Millipore), whose pH was adjusted using high-purity HCl and KOH as needed. At pH 8.0, a large, exponentially increasing repulsion was measured beginning at about 1000 Å from contact. A theoretical fit to the force profile,[8] based purely on electrostatic considerations, describes the data well and indicates that the layers are about 30% charged at this pH. The two bilayers can be compressed to a combined thickness of 109 Å, in good agreement with the space-filling model. No adhesion is observed even after very high compressions, and identical force curves are measured on repeated compression or expansion cycles.

(1) (a) Paleos, C. M.; Tsiourvas, D. *Adv. Mater.* **1997,** *9,* 695−710. (b) Khazanovich, N.; Granja, J. R.; McRee, D. E.; Milligan, R. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1994,** *116,* 6011−6012.
(2) Leckband, D. *Nature* **1995,** *376,* 617−618.
(3) (a) Leckband, D. E.; Schmitt, F.-J.; Israelachvili, J. N.; Knoll, W. *Biochemistry* **1994,** *33,* 4611−4624. (b) Moy, V. T.; Florin, E. L.; Gaub, H. E. *Science* **1994,** *266,* 257−259. (c) Lee, G. U.; Kidwell, D. A.; Colton, R. J. *Langmuir* **1994,** *10,* 354−357.
(4) (a) Pincet, F.; Perez, E.; Bryant, G.; Lebeau, L.; Mioskowski, C. *Phys. Rev. Lett.* **1994,** *73,* 2780−2783. (b) Berndt, P.; Kurihara, K.; Kunitake, T. *Langmuir* **1995,** *11,* 3083−3091. (c) Boland, T.; Ratner, B. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995,** *92,* 5297−5301. (d) Lee, G. U.; Chrisey, L. A.; Colton, R. J. *Science* **1994,** *266,* 771−773.
(5) (a) Berg, J. M.; Claesson, P. M. *Thin Solid Films* **1989,** *178,* 261−270. (b) Frisbie, C. D.; Rozsnyai, L. F.; Noy, A.; Wrighton, M. S.; Lieber, C. M. *Science* **1994,** *265,* 2071−2074. (c) Thomas, R. C.; Houston, J. E.; Crooks, R. M.; Kim, T.; Michalske, T. A. *J. Am. Chem. Soc.* **1995,** *117,* 3830−3834.
(6) Zasadzinski, J. A.; Viswanathan, R.; Madsen, L.; Garnaes, J.; Schwartz, D. K. *Science* **1994,** *263,* 1726−1733.
(7) Berndt, P.; Fields, G.; Tirrell, M. *J. Am. Chem. Soc.* **1995,** *117,* 9515−9522.
(8) Marra, J.; Israelachvili, J. *Biochemistry* **1985,** *24,* 4608−4618.
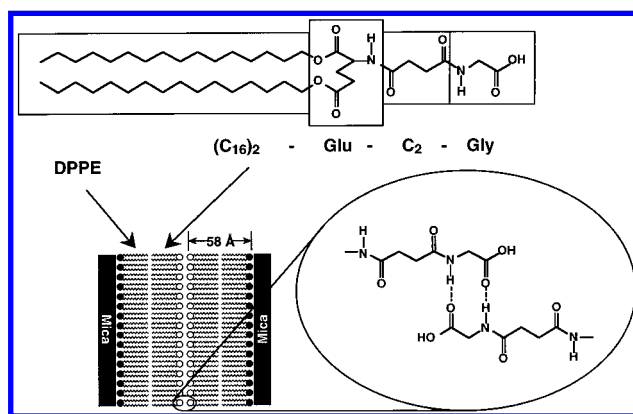
Figure 1. Structure of the $(C_{16})_2$-Glu-C_2-Gly amphiphile, and its location in the composite bilayer. The inset depicts the hydrogen-bonded dimers which form between $(C_{16})_2$-Glu-C_2-Gly amphiphiles in contact.
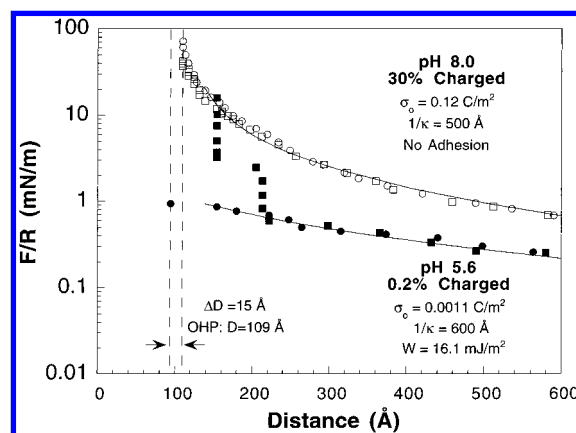
Figure 2. Force profiles for DPPE/$(C_{16})_2$-Glu-C_2-Gly bilayers in pure water at various pH 5.6 and 8.0. Circles and squares represent first and second approaches, respectively, at pH 5.6 (filled) and pH 8.0 (open). The zero of the distance axis corresponds to bare mica-bare mica contact. ΔD is the decrease in contact separation distance from pH 8.0 (109 Å) to pH 5.6 (94 Å). The line is a theoretical fit considering electrostatic double-layer forces,[8] with surface charge σ_o; Debye screening length l/κ, and separation of the charged surfaces ("OHP") listed.

After the pH is decreased to 5.6, a lower electrostatic repulsion is observed beginning at about 600 Å from contact; the electrostatic fit has these layers only 0.3% charged. At very short range, the surfaces abruptly "jump" together. A normalized pull-off force of $F/R)_0 = -74$ mN/m was required to separate the bonded surfaces. For comparison purposes, we calculate a corresponding adhesion energy per unit area[9] ($W = -F/1.5 \pi R$) of 16.1 mJ/m². We ascribe the moderately strong adhesion to the action of hydrogen bonds formed between the LB layers of glycine amphiphiles. Support for the existence of interlayer hydrogen bonding is provided by FTIR spectra for LB films of $(C_{16})_2$-Glu-C_2-Gly, which have spectral shifts indicating the formation of hydrogen bonds between amide groups.[10] Measurements made on layers of the same amphiphile without the terminal glycine (and no surface accessible amine group) show only a weak adhesion ($W = 2.0$ mJ/m²). Measurements made on layers of the methyl ester of $(C_{16})_2$-Glu-C_2-Gly have $W = 16.0$ mJ/m², suggesting that the terminal hydroxyl group is not required for the strong adhesion.

(9) Leckband, D. E.; Helm, C. A.; Israelachvili, J. *Biochemistry* **1993,** *32,* 1127−1140.
(10) Bandekar, J. *Biochim. Biophys. Acta* **1992,** *1120,* 123−143.

Table 1. Titration of the DPPE/(C$_{16}$)$_2$-Glu-C$_2$-Gly Bilayers[a]

pH	adhesion energy, W (mJ/m^2)	W attractive (mJ/m^2)	charged fraction (f)	probability unchd pair $(1-f)^2$	W, attr. per unchgd pair (kT)
6.0	15.6	16.1	0.022	0.956	1.6
6.8	11.4	13.2	0.054	0.895	1.4
7.6	8.1	11.7	0.13	0.757	1.5
8.0	0		0.30		0

[a] Force and adhesion data were obtained in 1 mM KBr to avoid ionic-strength-dependent pK_a shifts. Charged fraction (f) was evaluated as in Figure 2. The attractive part of the adhesion energy (W) was obtained by adding the maximum repulsive interaction energy observed during force measurements to the pull-off derived result.[3a]

The contact thickness of these adhered bilayers decreased to 94 Å at the lower pH. This 15 Å of additional rearrangement or interpenetration of the bilayers exposes amide groups to the interface for the formation of amine/carbonyl hydrogen bonds (Figure 1). At high pH, strongly repulsive forces prevent this interpenetration and resulting interfacial hydrogen bonding, maintaining the bilayer thickness at 109 Å.

Previously contacted adhesive surfaces could not be brought into smooth adhesive contact a second time, due to molecular-sized roughness on the surfaces (Figure 2). Apparently, portions of supporting bilayers are uprooted from the mica surface in order to maintain headgroup contact. The separated surfaces are roughened by this disruption, leading to additional repulsive forces which appear at about 120 and 60 Å from adhesive contact, the thickness of two and one bilayers, respectively.

The extent of charging of the layers smoothly increases as the layers are titrated between pH 5.6 and 8.0 (Table 1). Over the same range, a corresponding decrease in adhesion energy between the layers is observed. Considering the decreasing likelihood $(1-f)^2$ that two uncharged amphiphiles meet for hydrogen bonding as the uncharged fraction (f) decreases with pH, we calculate a fairly constant value for the adhesion energy per uncharged amphiphile pair of 1.5 ± 0.1 kT (pH 5.6 − 7.6). Because of the amphiphile uprooting, we cannot fairly compare this value to hydrogen-bonding energies, but it may be considered a lower bound.

During an SFA experiment, we can further load the bilayers after the initial contact, monitoring the change in contact area. The JKR theory[11] describes this relationship:

$$a^3 = \frac{R}{K}\left(P + 3\pi WR + \sqrt{6\pi WRP + (3\pi WR)^2}\right) \quad (1)$$

Here, a is the radius of the contact area, R is the mean radius of curvature, W is the adhesion energy per unit area, P is the applied load, and K is the elastic constant of the contacting bodies. A two-parameter fit of eq 1 can be made to a load profile, yielding K and W.

The load profiles for the (C$_{16}$)$_2$-Glu-C$_2$-Gly layers at pH 6.8 (Figure 3) show significant hysteresis. A two-parameter fit of eq 1 to the loading curve gives W = 0.14 mJ/m^2, much lower than the value calculated using pull-off force data (11.4 mJ/m^2). The comparably low adhesion energy measured on loading suggests that the surfaces are drawn into contact by relatively weak van der Waals interactions and hydrogen bonds are formed only after this contact has been made. As the surfaces are
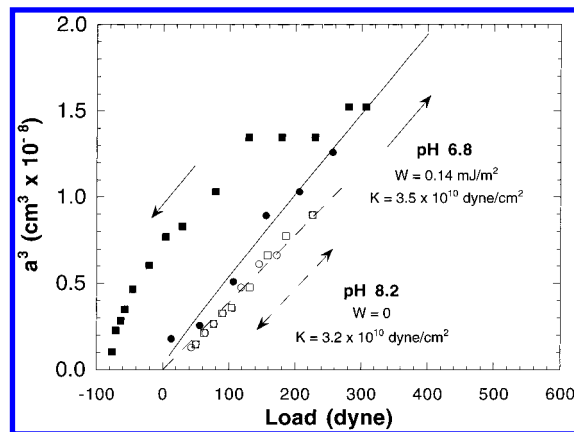
Figure 3. JKR adhesion analysis for DPPE/(C$_{16}$)$_2$-Glu-C$_2$-Gly bilayers showing pronounced hysteresis at pH 6.8. Loading data (filled circles) and unloading data (filled squares) are measured at the same spot (R = 1.5 cm). At pH 8.0, no adhesion hysteresis is observed between loading (open circles) and unloading (open squares) data (R = 1.3 cm). The solid line represents a fit of the loading data at pH 6.8. The broken line represents a fit of loading and unloading data at pH 8.0. K and W are derived from unweighted least-squares fits of eq 1.

unloaded, the hydrogen-bonded amphiphiles are uprooted from the membrane, leading to the high adhesion energy. At pH 8.0 (adhesion-free conditions), we observe no hysteresis in the load profiles (Figure 3). This observation precludes the possibility that plastic deformations in the glue, mica, or glass lens are responsible for the adhesion hysteresis.

We have demonstrated that hydrogen bonding between glycine headgroups can provide impressive structural support in supramolecular assemblies and, by extension, biological macromolecules. Indeed, upon separating hydrogen-bonded layers, the bilayers would rather sacrifice a strong hydrophobic anchorage of amphiphile tails[12] than sever energetically weaker hydrogen bonds between headgroups.[13] However, for surfaces separated under dynamic loading, the length over which fracture occurs must be considered.[14] While hydrogen bonds must be broken almost simultaneously, over a range of 4 Å or less, lipid pull-out can occur as a series of short, energetically inexpensive steps in which only a few methylene groups are exposed to water at a time. Our observations confirm that simple energetic considerations alone are not sufficient to predict the mode of fracture when biological macromolecules or membrane structures are subjected to realistic mechanical forces, due to this interesting competition between hydrophobic and hydrogen-bonding interactions. Such forces may be experienced in protein separation processes, detachment of cells from surfaces under flow conditions, or during circulation of liposomes in vivo.

(11) Johnson, K. L.; Kendall, K.; Roberts, A. D. *Proc. R. Soc. London A* **1971**, *324*, 301−313.

(12) Cevc, G.; Marsh, D. *Phospholipid Bilayers: Physical Principles and Methods*; John Wiley and Sons: New York, 1987.
(13) Vinogradov, S. N.; Linnell, R. H. *Hydrogen Bonding*; Van Nostrand Reinhold Co.: New York, 1971.
(14) (a) Bell, G. I. *Science* **1978**, *200*, 618−627. (b) Leckband, D.; Müller, W.; Schmitt, F.-J.; Ringsdorf, H. *Biophys. J.* **1995**, *69*, 1162−1169.