

Direct Measurement of Surface Forces between Monolayers of Anchored Poly(L-glutamic acid)

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Interactions between apposed poly(L-glutamic acid) monolayers in water were investigated by direct surface force measurements. The average degree of polymerization was 21. Surface force profiles consisted of a long-range electrostatic repulsion and a short-range steric repulsion. Secondary structures of the polypeptide were found to determine the short-range steric repulsion. The elastic compressibility moduli of the polypeptide monolayers were obtained to be 5.6 ± 2.2 MPa for the β_2 -structure-rich layer and 0.2 ± 0.1 MPa for the layer of ionized extended chains.

Direct measurements of the surface forces between apposed polymer layers in organic solvents at separations close to molecular dimensions have recently become possible by the use of the surface force apparatus.¹ When the surfaces of the apposed polymer layers approached a separation similar to that of molecular dimensions, the steric repulsions become predominant and, hence, measurable. In the case of polyelectrolytes, however, only limited kinds of polymers have been studied by the surface force apparatus previously.^{2,3}

Direct, nanometer range characterization of structures, mechanical properties, and interactions of well-defined polyelectrolytes on surfaces is essential for developing a new preparation of advanced materials, for obtaining a molecular level of understanding of the biological functions of proteins and cells, and for optimizing colloidal stabilizations.⁴ The Langmuir–Blodgett (LB) technique is well-suited for this purpose.⁵ First, modification of mica surfaces by two-dimensionally organized, well-defined functionalized groups is possible by simple deposition of polymer functionalized monolayers formed at the air–water interface. Second, the surfaces can be characterized by a large variety of physical chemical methods.

Taking advantages of LB methodology, we have launched systematic investigations for direct measurement of surface forces between various surfaces.⁵ Ionization and structural changes of poly(methacrylic acid) monolayers have been examined as a function of pH and added concentrations of salts.⁶ At shorter separation distances, apposed poly(methacrylic acid) monolayers behave like an oriented polyelectrolyte gel of two molecular thickness. This polymer, therefore, provides a simple model system to account for complicated properties of three-dimensionally networked polyelectrolyte gels. In the present work, we report our investigations on the surface forces between monolayers of anchored poly(L-glutamic acid), which forms various secondary structures through intra- and intermolecular hydrogen bonding (see Figure 1).⁷ Steric repulsions have been found between these layers and used to determine the elastic compressibility moduli of oriented, single molecular layers of polypeptides in various secondary structures. To our knowledge, this is the first report of a quantitative determination of

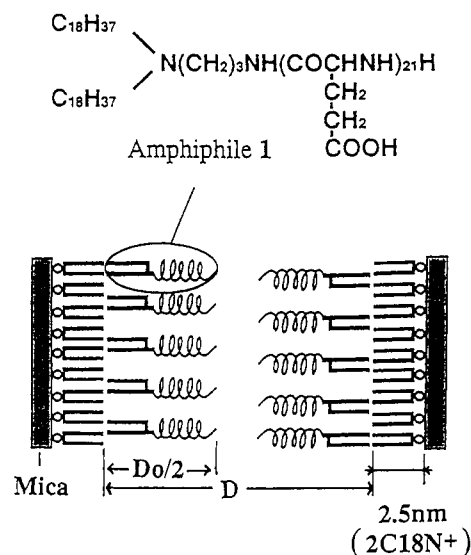


Figure 1. Schematic drawing of two interacting surfaces bearing anchored poly(L-glutamic acid) and a chemical structure of amphiphile 1.

mechanical moduli of oriented, monomolecular polymer layers and of their dependence on chemical structures of polymers.

Amphiphile 1 carrying the poly(L-glutamic acid) segment (degree of polymerization, $n = 21$) was prepared as described previously.⁷ Compound 1 formed a stable monolayer at the air–water interface as shown by a good surface pressure vs molecular area isotherm (USI system, FSD 50) (Figure 2). Upon compressing monolayer 1, different secondary structures such as α -helix and β -structures were formed depending on surface pressures.⁶ Fourier transform infrared (FT-IR) spectroscopy (Perkin-Elmer, System 2000) was used to study formation and orientation of secondary structures of the poly(L-glutamic acid) segment of 1. The transmission FT-IR spectra of LB films 1 (one layer) transferred onto CaF_2 plates were measured by using a TGS detector (resolution 4 cm^{-1}). Reflection absorption spectra of the films were obtained on Au-deposited (100 nm) glass by a MCT detector.

Figure 3A.a shows a transmission FT-IR spectrum of LB films 1 transferred onto CaF_2 plates at 25 mN/m and pH 5.6. The spectrum exhibits two major bands assigned to amide I at $1690\text{--}1600 \text{ cm}^{-1}$ and to amide II at $1520\text{--}1550 \text{ cm}^{-1}$. It

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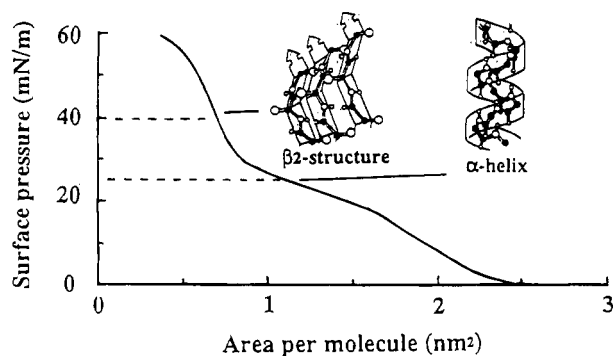


Figure 2. Surface pressure-molecular area isotherm of **1** over the pure water subphase (pH \sim 5.6) at 20.0 ± 0.1 °C.

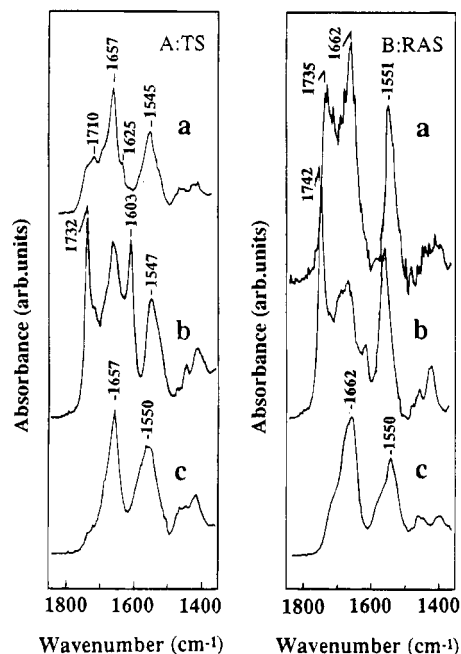


Figure 3. FT-IR transmission (series A) and reflection absorption (series B) spectra of LB films **1** transferred at 25 (a) and 40 mN/m (b) from the pure water subphase and that of LB film **1** transferred at 25 mN/m from the subphase at pH 9.6 (c).

displays a peak of amide I at 1657 cm^{-1} , ascribed to α -helix, and that at 1655 cm^{-1} , attributed to random coil.^{8–10} Appearance of the characteristic peak at 1603 cm^{-1} in a spectrum for the film deposited at 40 mN/m (Figure 3A.b) establishes the formation of a β_2 -structure upon compression,^{8,9} which is accompanied by disappearance of an α -helix peak and that of a random coil around 1655 cm^{-1} . The amide I band is deconvoluted into peaks of different secondary structures by assuming appropriate peak shapes¹¹ and is used to determine the orientation and the composition of each secondary structures from ratios of the transmission absorbance (A_T , Figure 3A.a,b) to the reflection absorbance (A_R , Figure 3B.a,b) of FT-IR spectra.¹² The percentages of amide bonds which are ascribed to α -helix, β_1 -structure, β_2 -structure and random coil (structureless) are estimated to be 5 (33°), 5 (60°), 4 (52°), and 86% (random) for the film prepared at 25 mN/m, where the numbers in parentheses are the average angles of the amide I transition moment to the vertical axis of the substrate.¹³ This ratio at 40 mN/m is 1 (29°), 2 (62°), 22 (67°), and 75% (random). The orientation angles of the amide I moments are about 30° for α -helix and 70° for β_2 -structure. It is well-known that the amide I transition moment of α -helix is oriented ca. 30° to the helix axis and that of β -structure is vertical to the molecular axis of the peptide.¹⁰ Therefore, α -helix orients its helix axis vertical

to the substrates, and β_2 -structure orients the molecular axis at ca. 20° to the vertical axis of substrates. The relatively short length of the peptide and the packing hindrance in monolayers must suppress the intensive growth of secondary structures, resulting in an apparently large content of random-coil structure of amides.¹⁴ Figures 3A.c and 3B.c are a transmission and a reflection spectrum of LB film **1** transferred at 25 mN/m from the subphase at pH 9.6 (KOH). The appearance of the carboxylate band at around $1560\text{--}1580\text{ cm}^{-1}$ at the expense of the carbonyl band at $1710\text{--}1740\text{ cm}^{-1}$ demonstrated dissociation of the carboxylic acid groups. From the decrease in the carbonyl peak intensities, we calculated the percentage of the carboxylic acid dissociation to be ca. 70%.

These secondary structures can be transferred onto hydrophobic mica surfaces at various surface pressures from the aqueous subphase to provide an oriented layer of anchored poly-(L-glutamic acid) in the desired conformation.¹⁵ LB deposition of dimethyldioctadecylammonium bromide ($2C_{18}N^+Br$, **2**) at 35 mN/m in the up-stroke mode was used to render the mica surface hydrophobic. Subsequently, these monolayer-covered surfaces were subjected to the direct measurement of surface forces (Figure 1). Force measurements between apposed monolayers on two cylindrical mica surfaces in water were carried out by using a surface force apparatus Mark4 (ANUTECH). Modified mica sheets on cylindrical lenses (radius $R \approx 20\text{ mm}$) were mounted as crossed cylinders in the apparatus. Procedures of the measurement were similar to those reported previously.^{6,16} Separation distances measured, D' , were those between the mica surfaces. For ease of discussing structural forces of **1** hereafter, these distances D' were converted to the distances between surfaces of the hydrophobic layers **2**, D , by subtracting twice the thickness of the layer of **2** (5.0 nm) from D' : $D = D' - 5.0\text{ (nm)}$. The measured force, F , was normalized by mean radius, R , of the surface curvature. This quantity is proportional to the free energy of interactions G_f per unit area between two flat surfaces according to the formula of Derjaguin approximation, $F/R = 2\pi G_f$.¹⁷ Differentiation of G_f gives the corresponding force per unit area, P , between two flat surfaces, $P = -dG_f/dD$.

Force-distance profiles, measured in pure water (pH \sim 5.6) for surfaces of LB films deposited at 25 (a) and 40 mN/m (b) from the pure water subphase, are shown in Figure 4. The profile at pH 9.6 for the surfaces prepared at 25 mN/m from the subphase at pH 9.6 (c) is also described in the same figure. In the first sample, as seen in Figure 3, the majority of peptides were in forms of α -helix and random coil, while in the second the β_2 -structure became dominant among second structures except the random-coil form.¹⁴ In the third sample, two-thirds of the carboxylic acid group dissociated.

Two major regions were clearly seen in the force-distance profile (Figure 4). At surface separations longer than 25 nm, typical double-layer electrostatic forces, due to ionized carboxyl groups, were observed. At separations shorter than 25 nm, the repulsion was found to be steric in origin and varied depending on the secondary structures existing in the surface layer. The decay length of the long-ranged component for the surfaces prepared at pH 9.6 (case c), 30 nm, agreed well with the Debye length (31 nm) of a salt concentration of added KOH of 10^{-4} M . This repulsion could be fitted by the diffused electronic double-layer theory¹⁸ by assuming the surface potential of 120 mV (the constant potential model).¹⁹ On the other hand, the decay length of the long-ranged repulsion at pH 5.6 was $100 \pm 5\text{ nm}$ for the surfaces prepared at 40 mN/m and $50 \pm 5\text{ nm}$ for that at 25 mN/m. These decay lengths were considerably smaller than those expected from the Debye length in pure water

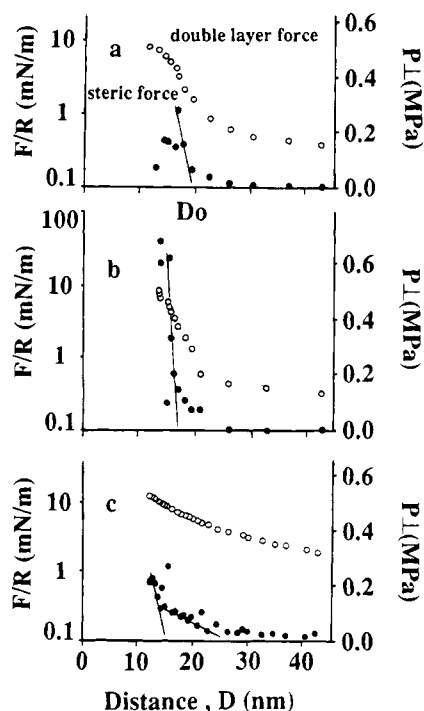


Figure 4. Surface force—distance (○) and stress—distance (●) profiles were measured in pure water (pH \sim 5.6) between apposed layers of poly(L-glutamic acid) amphiphile **1** transferred at 25 (a) and 40 mN/m (b) from the pure water subphase and those measured at pH 9.6 between layers deposited at 25 mN/m from the aqueous subphase at pH 9.6.

equilibrated by carbon dioxide (pH \sim 5.6) of 190 nm. This discrepancy could be rationalized as follows. It was difficult to determine zero force because of long decay length of repulsion at low salt concentrations.³ In addition, especially for the samples prepared at 25 mN/m and pH 5.6, which exhibited very weak repulsion, we often could not avoid inward and slow drift. This small drift does not cause any change in the steric repulsion discussed below.

The steric component of repulsion can be analyzed by differentiating the free energy of interaction G_f between two flat surfaces as shown in Figure 4, $P = -dG_f/dD = -(1/2\pi) \cdot (d(F/R)/dD)$. This procedure provides the stress—distance profile for deformation of the polypeptide layer in water, because the stress is an applied force per unit area, $-dG_f/dD$, needed to produce deformation. The stress curve increases when the steric component appears upon compression.²⁰ The sharpest increase is detected for surfaces prepared at 40 mN/m (sample with high content of β_2 -structure, Figure 4b). As surfaces approach, the stress curve displays the maximum at 16.2 ± 0.9 nm. We calculated the elastic compressibility modulus, $dP/(dD/D_0)$, to be 5.6 ± 2.2 MPa from the steepest slope (dP/dD , 0.31 ± 0.12 MPa/nm) of the stress—distance curve (see Figure 4b). Here D_0 is twice the initial thickness of deformed layer, which is obtained by extrapolation of the steepest curve to the zero stress, $D_0 = 18 \pm 1$ nm. This separation is close to the thickness 19.3 ± 0.5 nm of two molecular layers of the β_2 -structure, calculated by using the CPK model and the orientation angles obtained by FT-IR analysis. The carboxylic acid groups are known to interdigit within the β_2 -structure,⁸ which must cause a high compressibility of this layer. Slow increase from the onset (25 nm) of the stress should be ascribable to heterogeneity of the sample such as polydispersity in degree of polymerization, n . The stress curve for the sample prepared at 25 mN/m at pH 5.6 displays the onset at 25 nm, the maximum at 17 ± 1 nm, and plateau after the maximum (Figure 4a, case a). The plateau indicates a collapse and/or structural rearrangement of the poly-

(L-glutamic acid) layer, in agreement with poor reproducibility of force profiles upon repeating approaches. From the sharp slope, the compressibility modulus of the poly(L-glutamic acid) layer was calculated to be 1.5 ± 0.1 MPa. This value may be ascribable to β_2 -structure and/or α -helix, but heterogeneity makes the identification difficult. In case of the profiles at 25 mN/m and pH 9.6, we have observed two linear components in the stress profiles (Figure 4c). The elastic compressibility moduli were obtained to be 0.2 ± 0.1 MPa for the component with onset at ca. 25 nm and 1.2 ± 0.1 MPa for that at shorter than 15 nm. Transformation of the polypeptide chains should occur. These two moduli can be attributed to the extended and coiled chains of the polypeptide, respectively. The moduli of the polypeptide monolayers obtained for hydrogen-bonded secondary structures are in the same range as an elasticity measured for a typical rubber (1 MPa). On the other hand, the modulus for ionized extended chains is 1 order of magnitude smaller than that for a rubber.

Force profiles are only reversible during compression and decompression cycles in high alkaline conditions (pH $>$ 9). At lower pH of 5.6, weak pull-off forces were observed. The intensity of pull-off forces changed depending on the range how far two surfaces were pressed together. When the surfaces were brought in until the repulsion of 20 mN/m appeared, the pull-off force of ca. 4 mN/m was detected for the sample prepared at 40 mN/m (case a) and of ca. 1 mN/m for one at 25 mN/m (case b).

A significant correlation between the secondary structure of polypeptides, estimated by FT-IR spectroscopy, and the steric force, measured by the surface forces apparatus, is the most notable accomplishment in this work. The stress—distance profile measured by the surface force apparatus is discussed for the first time and is found to provide information on mechanical properties and conformational changes of secondary structures in water. One may note that mechanical properties discussed here reflect not only the intrinsic flexibility of polypeptide chains but also other effects such as osmotic pressure and combination of counterions with charged polypeptides. This work opens the door to study a wide range of structural changes and elastic properties of polymers in solvents, especially proteins and polyelectrolyte networks in water, at the molecular level by using the surface forces measurement.

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- (10) Peaks attributed to α -helix and random coil overlap each other. However, their half-widths are quite different, allowing us to deconvolute

the absorption band into these peaks.¹¹ Taking an example in Figure 3A.a, the half-widths of α -helix and random coil are 8 and 56 cm^{-1} .

(11) By comparing spectra of LB films which deposited at extreme conditions such as high pH, we obtained the position and half-width of peaks of each secondary structure in the amide I region. We assumed an appropriate peak shape to be the product of a Gaussian and Lorentian function.

(12) The transmission method emphasizes vibrational modes parallel to the film plane, and the reflection emphasizes those perpendicular to the surface. This enables us to calculate orientational angles of vibrational transition moments: Umemura, J.; Kamata, T.; Kawai, T.; Takenaka, T. *J. Phys. Chem.* **1990**, *94*, 62.

(13) The errors in orientational angles of secondary structures are $\pm 3^\circ$ except that ($\pm 5^\circ$) of α -helix in the film prepared at 40 mN/m. The considerably large error in the latter value is caused by small content (1%) of α -helix.

(14) Apparent large contents of random coil structures are caused (at least partially) by limited growth of secondary structures. For example, when three molecules form a perfect β_2 -structure, one-third of the amide bonds are in free terminal bonds and remained in random-coil structures.

(15) Transfer ratios of monolayer **1** were 1.0 ± 0.1 at a surface pressure of 25 mN/m and 0.9 ± 0.1 at 40 mN/m. Following observations indicated that compositions of secondary structures at the air–water interface were likely to be maintained in LB films on mica exposed to water, although we could not measure spectra of films *in situ* on mica exposed to water at the moment. FT-IR spectroscopy demonstrated that LB films **1** deposited on

dry CaF_2 and Au-deposited plates were basically identical, independent of the kind of substrates. When they were immersed in water and then dried, these films on CaF_2 and Au-deposited plates exhibited identical spectra to those taken before immersion, showing that secondary structures should be stable in water. Good correlation of measured force profiles with secondary structures determined by FT-IR is additional support for the stability of secondary structures.

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(19) In data curve fitting, the charged surface (zero separation in the double-layer model) was assumed to locate at a separation (D) of 23 nm from the mica surface. This separation was chosen from the stress–distance profile, where the stress began to increase sharply at this separation upon compression.

(20) In the case of certain samples, the force–distance (more visibly the stress–distance) curves changed during repeating compression–decompression cycles, indicating some rearrangements of secondary structures. Therefore, the force profiles discussed were those taken at the first approach unless stated otherwise.

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