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Normal and Shear Forces between Mica and Model Membrane Surfaces with Adsorbed Hyaluronan

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ABSTRACT: Using a surface forces apparatus, we have measured the normal and shear forces between surfaces containing physisorbed hyaluronic acid (HA), which is a major component of synovial fluid and which is believed to play a role in joint lubrication. The adsorption mechanisms involved the ionic binding of negatively charged HA (i) to positively charged surfactant bilayers supported on mica substrates and (ii) to negatively charged mica surfaces via Ca^{2+} bridges. Our results show that *physisorbed* HA fails as a lubricant when surfaces are pressed strongly together and/or sheared due to the squeezing out of the HA from between the surfaces. On the basis of these and previous studies, we conjecture that HA might function as an effective boundary biolubricant, exhibiting both low friction and low wear, but only if it is chemically or specifically bound exclusively to each surface, but not to both surfaces.

Introduction

Hyaluronic acid (HA) is a long linear negatively charged biological polymer or “polyelectrolyte” (see Figure 1a) which is present at high concentrations in synovial fluid. It is believed to play an important, but as yet incompletely understood, role in joint lubrication, and in certain cases it is injected into the joints of patients suffering from arthritis.[1] However, despite research going back many decades, its mode of operation as a biolubricant and other possible physiological functions are still far from clear,[2−5] and theories of polyelectrolyte-mediated lubrication are proving to be highly complex and still in their infancy.[6] Possible roles are (i) that HA modifies the rheological (viscous and viscoelastic) properties of bulk synovial fluid,[7] (ii) that it is directly responsible for lubrication (hydrodynamic or elasto-hydrodynamic) even when it is present in the bulk,[8] (iii) that it functions as a boundary lubricant,[9] or (iv) that it serves as a transporter for certain phospholipids in the synovium that do the actual lubrication.[10] Understanding the physiological role of HA has obvious applications for detecting and treating arthritis and other disorders where the biolubrication system has broken down, leading to cartilage and bone damage. Such disorders are very common, causing loss of joint function and persistent pain, and most cannot yet be satisfactorily treated, let alone cured.

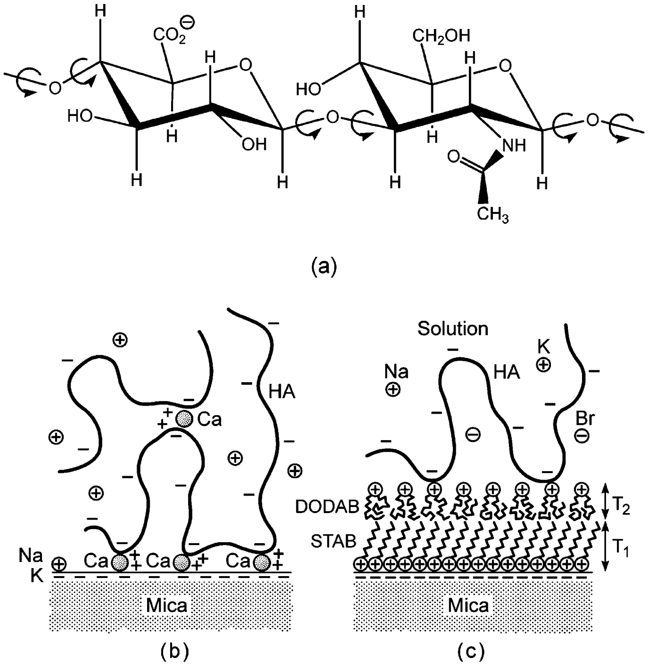

Figure 1. (a) Chemical structure of a repeating unit of hyaluronic acid, showing the location of the negative charge and six rotational bonds per unit. (b) Mode of adsorption of negatively charged HA onto negatively charged mica surfaces via divalent positively charged calcium bridges and (c) onto positively charged surfactant bilayer surfaces via direct electrostatic binding. The counterions in solution are the Na^+ ions that have dissociated from the HA and HEPES buffer, Ca^{2+} and Cl^- ions from the $CaCl_2$, Br^- from the surfactant, and some K^+ ions that have dissociated from the mica surfaces. Each system is overall electroneutral. The outer monolayer shown in (c) is DODAB, but experiments were also conducted with STAB as the outer layer. In all subsequent plots of forces vs distance, we adopt a convention where D corresponds to the mica−mica surface separation, so that the bilayer surfaces are separated by $D - 2(T_1 + T_2)$.

In a previous study,[7,11] we investigated the way HA functions as a lubricant “additive” under conditions when it is present in the bulk solution at high concentrations but does not adsorb to the smooth negatively charged mica surfaces used in that study. Our results were consistent with polymer theory and we discussed their implications for understanding how nonadsorbing HA would be expected to behave in synovial fluid. One of our conclusions was that if HA is to be an effective biolubricant, it must be attached to the surfaces that it is lubricating and/or protecting, especially if these surfaces are subjected to high compressive pressure as occurs in joints. There are two ways whereby a charged polyelectrolyte such as HA can be “attached” to a surface: via physisorption or chemisorption. The first involves physical bonds such as van der Waals, hydrophobic, hydrogen-bonding, ionic, or zwitterionic (dipolar) bonds. The second involves chemical bonds or strong complementary (ligand−receptor or lock-and-key type)

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bonds. The former tend to be nonspecific, for example, a physisorbed polyelectrolyte molecule will generally bind to both surfaces, whereas the latter tend to be "site-specific". It is also worth noting that specificity does not necessarily imply strength in a complementary bond.[12]

In the case of HA, its mode of attachment, if any, to the cartilage surface is unknown; it may even become trapped in the 2−6 nm sized pores on the cartilage surface[13] rather than bind to it. Even weak physisorption can lead to good boundary lubrication, provided the coverage is high and the applied pressure is low.[14] In this paper, we describe how HA functions as a biolubricant when it is physisorbed nonspecifically to mica and to model membrane surfaces.

Experimental Section

A surface forces apparatus (SFA) was used as previously described for measuring normal[15] and lateral or shear[16] forces. The SFA method enables simultaneous measurements of both of these forces at the same time as the surface shape (e.g., the contact area) is continually monitored, and the separation between the two interacting surfaces is measured to an accuracy of 1 Å, using an optical multiple-beam interference technique where fringes of equal chromatic order (FECO) are viewed in a spectrometer. The positions and shapes of these fringes (see Figure 3) allow one to measure the absolute thicknesses of adsorbed monolayers, bilayers, or membranes[17−19] and to distinguish between partial (depleted) and fully developed lipid monolayers or bilayers.[20] In this study, the supporting substrate surfaces of mica were either untreated or had surfactant bilayers deposited on them. This was done to mimic the lipid bilayers of biological membranes that are usually, but not always, found at the surfaces, interfaces, and junctions of cells and biological tissues. The mica sheets were initially curved and placed in the "crossed-cylinder" configuration in the SFA chamber. Their (undeformed) cylindrical radius of each surface R was typically 1−2 cm, but under a large compressive force or pressure the two surfaces would flatten and conform to each other. The radius r of the flattened area varied from 1 to 50 μm, depending on the applied load, and could be directly visualized and measured using the optical technique.[21,69] In the case of flattened surfaces, their contact area may be defined as $A = \pi r^2$.

Two types of electrostatic binding (adsorption) were tried (Figure 1b,c): (i) adsorption of negatively charged HA to positively charged (bilayer) surfaces and (ii) adsorption of HA to negatively charged mica via calcium ion bridging.[22] In the latter case, $CaCl_2$ was simply added to the HA bathing solution. In the case of the bilayers, their outer monolayers were either in the fluid state (the more common state of biological membranes) or in the frozen state (below the so-called lipid chain melting temperature). For both bilayer systems studied, the first (inner) monolayer was a frozen layer of octadecyltrimethylammonium bromide (C_{18}-TAB or STAB[23]), which was deposited onto the molecularly smooth mica surfaces by adsorption or "self-assembly" from solution.[24] The second (outer) monolayer was either another layer of STAB or a layer of dioctadecyldimethylammonium bromide (diC_{18}-DAB or DODAB), again adsorbed from solution. The outer STAB layer was in the frozen state, exposing a positively charged $-N^+(CH_3)_3$ group, while DODAB was in the fluid state, exposing $\rangle N^+(CH_3)_2$ groups. The negatively charged carboxyl groups −COO− of HA were expected to bind to both of these cationic groups.

High-quality clear ruby muscovite mica was obtained from S&J Trading Inc.; STAB (99% pure) was obtained from Sigma (product no. 35924-6), DODAB (99.5% pure) was obtained from Kodak (product no. 1354307), and $CaCl_2$ was obtained from Fisher Scientific as $CaCl_2{\cdot}2H_2O$ (product no. C-79-500) with $CaCl_2$ assay of 74−78%. Two kinds of HA were used: one was obtained from Biotechnology General (Israel) and one from Sigma, and both were used as received. The HA (Sigma, H-9390) was the sodium salt of hyaluronic acid (Figure 1a) from *streptococcus zooepidemicus*, of viscosity-average molecular weight of 540 kDa, an "end-to-end" distance $\langle s \rangle$ of about 156 nm, and a radius of gyration R_g of 64 nm.[7] This HA was used only for the experiments with the Ca^{2+}−mica system (Figure 1b). The HA from Biotechnology General was of viscosity-average molecular weight of 1.6 MDa, an "end to end" distance of about 300 nm, and an R_g of 122 nm. This HA was used for the experiments with the surfactant bilayers (Figure 1c). The two HA samples were of the highest purity available although the nature of the impurities, for example, whether protein, lipid, or other polymers, is unknown. A full account of the relevant properties and characterization of the HA used was given in ref 7, and in ref 11 a theoretical analysis of its interaction forces agreed well with the measured forces, suggesting that the samples are fairly pure.

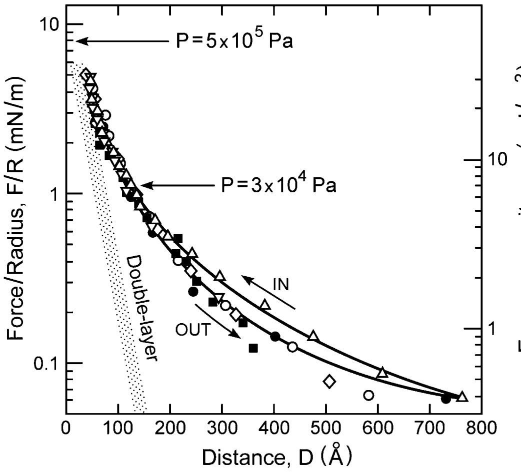


Figure 2. Force F vs separation D between two curved mica surfaces of radius $R \approx 1-2$ cm in a 2 mM $CaCl_2$ Hepes buffer solution containing 3.3 mg/mL HA of MW 540 kDa. The six different symbols refer to six different runs at three different "contact positions". The right-hand ordinate shows the corresponding interaction energy per unit area between two flat surfaces, based on eq 1. The local measured pressures P at the junction were calculated using eq 2. The shaded band shows the maximum double-layer force based on theory and previous experiments.

HA solutions were made in a HEPES buffer, N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (Sigma, H-7523; used as received). 2.383 g/L of HEPES powder was titrated with NaOH solution to obtain a final solution of 4.13 mM Na^+ at pH 7.4.

Results shown are based on six different experiments (different pairs of mica sheets) as well as on different contact positions within an experiment. In all, 19 force curves are presented, including two control runs (without HA in the solution).

Unless stated otherwise in the text or figure legends, all HA solutions were 3.3 ± 0.1 mg/mL.

Results

A. Indirect Adsorption of HA on Mica via Ca^{2+} Bridging. 1. Force Measurements.

Figure 1b shows the way HA segments bind to negative surface charges via Ca^{2+} bridges to form a weakly bound layer of adsorbed HA molecules on the mica surface. Figure 2 shows the measured force F vs the separation D between two mica surfaces each of (cylindrical) radius R immersed in an aqueous 4.13 mM $Hepes^-{-}Na^+$ buffer solution containing 3.3 mg/mL HA[25] and 2 mM $CaCl_2$. According to the so-called "Derjaguin approximation",[26] the corresponding interaction energy E per unit area between two flat parallel surfaces is given by

$$E(D) = F(D)/2\pi R \tag{1}$$

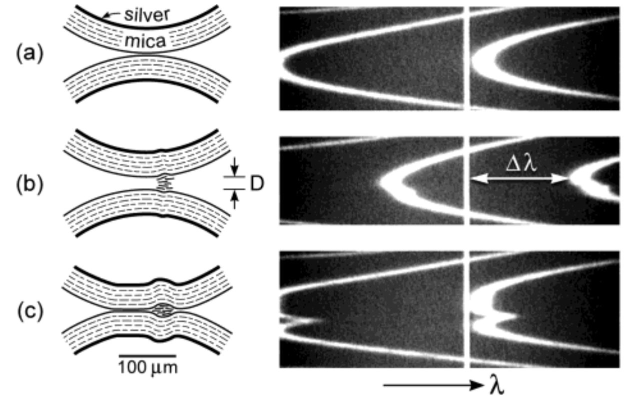


Figure 3. Surface geometry (left) and corresponding fringes of equal chromatic order (FECO) (right). (a) Picture taken as the surfaces were being rubbed against each other just before they became damaged. The two mica surfaces are quite strongly compressed at a surface separation of $D \approx 2$ nm (but are still rounded). Still, the friction between the surfaces was below our detection limit. (b) Surfaces become damaged after 45 s of sliding in the configuration shown in (a): mica flakes have detached from some regions on the surfaces and accumulated in regions near the area of closest approach, pushing the surfaces apart (fringes shift to larger wavelength λ) and locally bending the mica sheets (fringes no longer uniformly curved). (c) FECO at $t = 105$ s after the surfaces have been forced back into contact at $D \approx 2$ nm as in (a). The intrusion on the fringe is much more pronounced since the trapped mica flakes force the mica sheets to bend further.

from which the corresponding pressure P (force per unit area) between two flat surfaces can be obtained by a straightforward differentiation:

$$P(D) = -\mathrm{d}E/\mathrm{d}D = -(\mathrm{d}F/\mathrm{d}D)/2\pi R \qquad (2)$$

In the case of elastically flattened surfaces, the pressure is obtained directly from $P = F/A$, where A is the measured contact area.

For surface potentials in the expected range[2] −50 to −100 mV, the maximum double-layer force[11] between two mica surfaces in 4.13 mM $\text{Hepes}^- - \text{Na}^+$ buffer solution containing 2 mM $CaCl_2$ and 6.8×10^{-3} mM $\text{HA}^- - \text{Na}^+$ (8.5 mM dissociated Na^+ ions), as expected theoretically[27] and previously measured,[28] is shown by the shaded band in Figure 2. It is clear that the measured repulsion exceeds this force, especially at large separations. In our previous study with nonadsorbing HA in monovalent Hepes^-–-Na^+ solution without calcium, the measured forces were pure double layer at all separations, slightly modified by the additional HA counterions in the solution, which *reduced* the range of the repulsion. The *increased* magnitude and range of the repulsion measured in $CaCl_2$ solution clearly indicate that HA has adsorbed to the surfaces via Ca^{2+} bridging. However, the adsorption is weak and of low density: the additional force or pressure P is small, and its range is between 1 and 2 radii of gyration of the molecule ($R_g \sim 640$ Å), which is expected for a loosely adsorbing polymer.[27] Moreover, the repulsion appears to merge with the theoretically expected double-layer force at smaller separations (pressures above $\sim 2 \times 10^5$ Pa or 2 atm), which suggests that the HA is being squeezed out from between the two surfaces as they are pressed together. The measured forces in the presence of HA are clearly more repulsive at all the separations studied, although it is difficult to quantify exactly by how much because there is no complete theoretical prediction for the force−distance function of such interactions.

There are good reasons to expect only a weak adsorption of HA on mica via calcium bridging: calcium—and to an even greater extent magnesium—is a small "hydrated" cation[29] that does not readily lose its water of hydration on binding to certain anionic groups or surfaces, such as mica.[30] This means that the ionic binding is mediated by a water molecule and is, consequently, weak. Since the calcium ion is itself mediating the binding of HA to the surface, we may have two weak bonds in series, resulting in an even weaker bond. Although calcium is known to strongly bind to carboxylic acid groups[31] and to cause strong interbilayer (headgroup−headgroup) and intrabilayer (adhesive) bridging forces between bilayers composed of the common negatively charged lipid phosphatidylserine,[32−35] turbidity measurements of bulk the HA solutions (not shown) reveal a very small effect of calcium on the NTU (Nephelometer turbidity unit), which increased from ∼2.0 to ∼3.5 in the range from 0 to 140 mM $CaCl_2$ (in comparison, in the absence of HA in the aqueous solution the NTU value was ∼0.03), indicating that no significant aggregation occurs *in bulk solution* due to calcium bridging. These findings are consistent with the weak binding of HA to mica via Ca^{2+} bridges, discussed above.[36] The results of Figure 2 are likely to be due to HA being squeezed out, or to slow "bounce back" kinetics, since for each run the forces measured as the surfaces were compressed were larger than the force measured when the surfaces were decompressed.

2. Shear Measurements. Previous friction force measurements[7] of nonadsorbing HA found that HA does not function as a good boundary lubricant even though the increased bulk viscosity imparts some lubrication advantages. The measured shear (frictional) forces between two mica surfaces in HA solution with $CaCl_2$ were below our detection limit of 2 μN throughout the measurements, i.e., at all loads, up to the point at which the mica surfaces became damaged. The fact that the friction was below our detection limit suggests that HA may act as a good boundary lubricant, at least at low loads or pressures. When the shearing surfaces were compressed to a normal force of $F/R = 8$ mN/m, corresponding to $P = 5$ atm (cf. Figure 3), damage occurred and the friction force went up. At this pressure, the surfaces were still curved; i.e., no elastic flattening of the surfaces could be observed. The surfaces could be sheared at this pressure for about 1 min before damage occurred. Clearly, the fact that the damage did not occur immediately suggests that the polymer was being squeezed out of the gap between the surfaces during this period, but that as long as it remained between the surfaces, it served both as a low friction lubricant and as a protecting cushion for them. Whereas cartilage may be more or less sensitive to damage than mica, this still suggests that HA can protect the cartilage as long as it remains between the surfaces. The damage was probably caused when all the polymer was squeezed out of the gap and the two mica surfaces touched each other. In some experiments, the damage appeared to occur at the center; in others, it occurred a bit off center. An image of the fringes before and after damage is shown in Figure 3 together with the corresponding shape of the surfaces in a case where damage occurred off center.[37] As shearing progressed (under the same load and sliding velocity), the damage eventually spread to all regions of the "contact area", during which time the initially smooth surfaces became rough and the friction force steadily increased.

B. Direct Adsorption of HA onto Positively Charged Surfactant Bilayers. Since free Ca^{2+} failed to produce good lubricity or protect the surfaces from damage at high loads, we decided to reverse the charge on the surface, thereby allowing for *direct* ionic binding to occur to positively charged groups on the surfaces. In addition, the surfaces were modified to more closely mimic biological membrane surfaces. This was done by having a bilayer of cationic surfactant deposited on each mica surface, as shown in Figure 1c. Two different outer layer surfactants were used with the same inner layer surfactant. The inner layer was STAB, which was deposited as described in the Experimental Section. The thickness of two such layers in contact, as measured from the FECO fringes[17−19] on several samples, was $D = 2T_1 = 27.5 \pm 2.5$ Å (where $D = 0$ refers to bare mica−mica contact), giving $T_1 = 13.8 \pm 1.2$ Å per layer (Figure 1c). This value is within the experimental error of a previous measurement[24] but slightly more accurate due to the larger number of measurements (smaller error). This layer was characterized earlier,[24] and it was shown to expose a hydrophobic surface composed of $-CH_3$ and $-CH_2-$ groups. Given the molecular volume $v = 625$ Å^3 of STAB (see pp 112 and 316 in ref 38), the measured thickness of $T_1 = 13.8 \pm 1.2$ Å corresponds to an area per molecule of $625/13.8 = 45 \pm 3$ Å^2. This is precisely the area per negative charge on the mica lattice,[39] indicating (i) that the STA^+ headgroup has fully neutralized the mica surface on adsorption and (ii) that the hydrocarbon chains are in the close-packed, solid crystalline (frozen) state. This further implies that the chains (whose cross-sectional area is ∼20 Å^2 per chain) must be at an angle to the surface, as shown schematically in Figure 1c.

A second monolayer was then deposited onto the first monolayer, which now adsorbed with its hydrophobic tails facing “down” and its hydrophilic heads facing “up” to the aqueous solution. This second monolayer was either DODAB, as shown in Figure 1c, or another monolayer of STAB (which is not shown in the figure). DODAB adsorbs in the fluid state due to the inability of its hydrocarbon chains to align parallel to each other, while STAB adsorbs in the frozen state.

1. Forces between Fluid DODAB Outer Monolayers in HA Solutions. The outer DODAB layers were allowed to adsorb (self-assemble) onto the STAB monolayers from a few grains of DODAB dispersed in the aqueous solution. The measured forces between these bilayers in water (strictly a dilute solution of DODAB) and in HA solution are shown in Figure 4. After >20 h, the bilayers were found to have a thickness of $D = 2(T_1 + T_2) = 46$ Å, as ascertained from their thickness when in adhesive contact (see top of Figure 4b). This corresponds to a DODAB single layer thickness of $T_2 = (46 - 2T_1)/2 = (46 - 27.5)/2 = 9.3$ Å, which is clearly an incomplete layer. The normal forces reveal a long-ranged exponentially repulsive double-layer force (Figure 4a), as expected from the DLVO theory.[40,41] In the absence of HA the double-layer force is well-fitted by a surface potential of 54 mV, corresponding to a surface charge density of 10^{-3} C/m^2 (one electronic charge per 160 nm^2) and a Debye length of $\kappa^{-1} = 420$ Å, which corresponds to a ∼50 μM 1:1 electrolyte solution. These are physically reasonable values.

But the measured attractive short-range component is much stronger than expected from the (theoretically expected) van der Waals force. Thus, both with and without HA the surfaces jumped into adhesive bilayer−bilayer contact (at $D = 46$ Å) from a separation of about $D = 145$ Å—a jump of 99 Å, which is significantly greater than that expected from the van der Waals force.[27] This behavior is characteristic of incomplete (laterally stressed) bilayers that expose excess hydrophobic methylene groups of their lipid chains which gives rise to an additional “hydrophobic” attraction.[42,43]

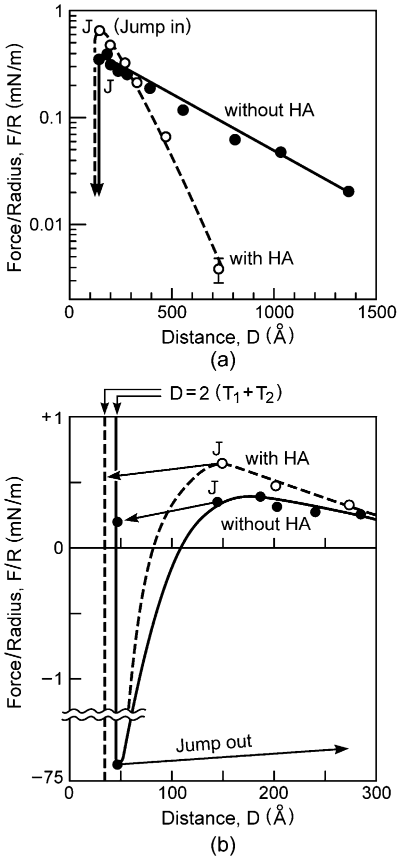


Figure 4. Long-range double-layer and short-range attractive van der Waals and hydrophobic forces measured between two mica surfaces coated with self-assembled DODAB-on-STAB bilayers in water before and 1.5 h after addition of HA. In both cases the short-range attraction and final adhesion forces were greater than expected from the van der Waals force alone. (a) Repulsive forces plotted on a log−linear scale. Spontaneous jumps into adhesive contact occurred from the force maxima at points J. (b) Forces at D below 300 Å plotted on a linear scale showing the jumps J into adhesive contact at $D = 2(T_1 + T_2)$. The solid vertical line is the DODAB−DODAB contact before adding HA, and the dashed vertical line is the contact after adding HA.

After addition of HA to the solution, the repulsive double-layer force had a shorter decay length of 113 Å (Figure 4a), which is only slightly shorter than the theoretical Debye length of *nonadsorbing* HA of $\kappa^{-1} = 120$ Å.[11] Assuming the same location of the outer Helmholtz plane (OHP),[44] the measured force is well-fitted by a surface potential of 76 mV, corresponding to a surface charge density of 6.5×10^{-3} C/m^2 (one electronic charge per 25 nm^2), which is 6 times higher than that measured without HA. However, this is only the “apparent” charge density, for when HA adsorbs the OHP is pushed farther out (by a distance corresponding to the average thickness of the adsorbed HA layer) and becomes diffuse. The real charge density must therefore be lower.

Interestingly, after the addition of HA, the short-range van der Waals and hydrophobic attraction *increased*: as shown in Figure 4b, the jump distance into adhesive contact increased from 145 to 150 Å even though the jump occurred from a more repulsive force, and the contact separation at $D = 31.6$Å had moved *in* by 14.4 Å; i.e., the outer DODAB monolayers had thinned by 7.2 Å per layer. This shows that the HA had removed most or all the DODAB molecules from the outer monolayer before or during the approach of the surfaces into contact. This observation lends support to the hypothesis[10] that HA helps in transporting phospholipids such as phosphatidylcholine[45] in synovial fluid, since it is clear that it was the HA that caused the DODAB amphiphiles to be removed from the surface layers into the solution.

We note that throughout these experiments a few grains of DODAB were always present in the water. Thus, the solution was saturated with the surfactant (at its critical micelle concentration, which is very low for double-chained surfactants[27] and well below the HA concentration in the solution), and so there would be no tendency for the surfactant to leave the bilayer under natural conditions. Indeed, the reverse is expected, since it was these grains from which the DODAB diffused to the STAB surfaces in the first place.

The increased attraction may also be due to HA bridging which, as discussed above, generates an additional attractive "bridging" force between two surfaces. It is possible that as the surfaces are pulled together by these forces, the HA−DODAB complexes form micelles that are squeezed out into the bulk solution. This would imply that the energy change of forming these micelles together with the increased hydrophobic energy of adhesion is overall favorable.

2. Forces between Frozen STAB Outer Monolayers in HA Solutions. The ease with which HA removed the outer layer surfactant when in the fluid state suggested that a more robust bilayer or substrate surface may be required for effective adsorption. To achieve this, a *frozen* layer of STAB, in contrast to the *fluid* layer of DODAB, was deposited on the first STAB layer (Figure 1c). The second layer of STAB was found to adsorb (self-assemble) quickly and reach full coverage overnight.

The STAB−STAB bilayer thickness was now measured to be $T_1 + T_2 = 34 \pm 1.5$ Å ($D = 68 \pm 3$ Å for two bilayers in contact; data not shown). Since the inner monolayer thickness was previously found to be $T_1 =$ 14 Å, the outer STAB monolayer thickness was $T_2 =$ $34 - 14 = 20$ Å, which implies that the area per molecule is $625/20 = 31$ Å^2. This is close to the van der Waals area of ~36 Å^2 occupied by the trimethylammonium group, and it indicates that the molecules in the outer monolayer are as close-packed as they can be.[46] The chains are still tilted to the normal because of the lower cross-sectional area of a methylene chain (~20 Å^2), but not as much as in the inner layer (Figure 1c) where the area per molecule of ~45 Å^2 is determined by the electrostatic interaction of the STA$^+$ group with the negative O$^-$ groups on the mica surface. The close-packed headgroups also imply that the positively charged bilayer surface is fully hydrophilic.

Figures 5 and 6 show the measured forces between two STAB−STAB bilayers in water and in HA solutions. The latter were found to be highly time- and history-dependent after the introduction of the HA into the solution, indicative of slow adsorption (binding) and rearrangements within the adsorbed layers, as well as various relaxations occurring both during and after the approach and separation of the surfaces.

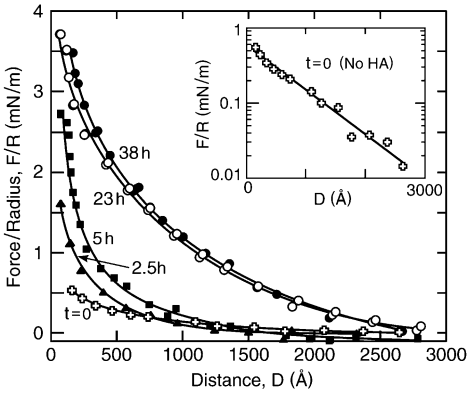


Figure 5. Details of force runs (on second approach) between STAB bilayers at different times after injection of HA. The repulsion appears to saturate after about 1 day. Inset: long-ranged, exponentially repulsive double-layer force in water before addition of HA, shown on a semilog plot. The Debye length is 740 Å, and the surface potential is +85 mV, which corresponds to unit surface charge per 130 nm^2.

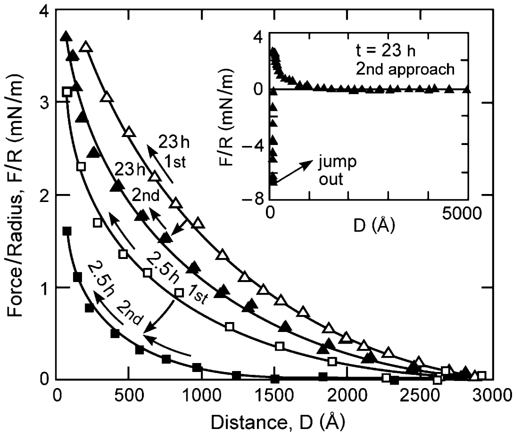


Figure 6. Details of forces on first and immediate second approaches between STAB bilayers in water measured 2.5 and 23 h after HA addition into the solution (second approaches typically measured ~10 min after the first). Saturation adsorption is reached after ~1 day, after which the force ceases to increase further (cf. Figure 5). Inset: adhesion force measured after 1 day (at saturation adsorption) after compressing the surfaces all the way to flattened bilayer−bilayer contact under a large compressive force. If the surfaces are separated before they reach contact, there is no adhesion.

Figure 5 shows the measured forces in pure water as well as at different times after HA was introduced into the solution at $t = 0$. In water with no HA added (crosses) the force followed a long-ranged exponentially decaying repulsion (see inset) as expected for the double-layer force between two cationic bilayers in dilute aqueous solutions.[47] After addition of HA the repulsion grew steadily outward with time, reaching a plateau after about 24 h, indicative of saturation adsorption. It is interesting to note that for the first 2 h after the commencement of adsorption the repulsion increases at short range (below about 750 Å) but *decreases* at larger separations. This is attributed to the initial adsorption of HA in a configuration that corresponds to its coiled state *in solution*, i.e., extending about R_g and giving rise to a steric repulsion that is of similar range. At larger separations the force is determined by the double-layer repulsion, which is now *weaker* due to the reduced Debye length arising from the increased concentration of sodium counterions in the solution (cf. Figure 4a) and hence the overlap in the forces at some finite separation. With further adsorption ($t > 5$ h), the steric repulsion eventually grows to dominate over the double-layer force at all separations, and—as in past studies with adsorbed water-soluble polymers on charged surfaces—the ultimate range of the force greatly exceeds the radius of gyration, R_g, of the molecules.[48−52]

The magnitudes and nonexponential shapes of the force profiles show them to be due to a combination of the steric and electrostatic repulsions between the overlapping negatively charged coils.[53] The slowness of the approach to saturation adsorption on the surfaces was reflected in other slow relaxation processes, for example, the reexpansion of the polymer layers after they are compressed during a force run. Thus, as shown in Figure 6, the forces on a first approach were always higher than on the second (and subsequent) approaches when these were done within a few minutes after the (first) separation—an effect that has been seen before with other adsorbed high-MW polymers.[14,48−56] In those studies, this effect was attributed to the slowness with which the compressed polymer layers “bounce back”, i.e., to their slowness to disentangle and break the new segment—surface bonds that had been formed during the compression. However, in the case of the compressed HA layers, a totally different mechanism appears to be operating that has important implications for its lubricating properties. First, we note in Figure 6 that in all the compressions the surfaces came into bilayer—bilayer contact under a fairly weak force or pressure corresponding to a few atm at most. This is quite different from the behavior of adsorbed polymers such as polystyrene, poly(ethylene oxide), and others[14,48−56] in various solvents, both aqueous and nonaqueous, where the compressed polymer layers were not forced out even under much higher pressures but became increasingly more compacted and stiff. In contrast, the HA layers were squeezed out with remarkable ease, allowing the STAB bilayer surfaces to come into adhesive contact at the same separation D as before the HA was introduced into the solution.

We conclude, therefore, that the reason for why the second compressions were always less repulsive than the first is not because the compressed polymer takes a finite time to “bounce back”, but because the polymer is “squeezed out” of the gap. The “relaxation” time for the force to return to its full value is therefore determined by the rates at which HA diffuses back into the gap and readsorbs onto the surfaces rather than by the rate at which the compressed layers reexpand. The physiological implications of the unusually easy slippage of HA between two highly confined surfaces are discussed in the Discussion.

Unlike the DODAB bilayers, the more rigid STAB—STAB bilayers appeared to remain intact during repetitive force runs in HA solution: the mica—mica separation remained constant at $D = 68 \pm 3$ Å, showing that the HA did not remove STAB molecules from the outer layer. The inset in Figure 6 shows the short-range adhesion force measured after two STAB bilayers are pressed together after all the HA has been squeezed out from between them. The adhesion force is larger than expected from a pure van der Waals attraction and could be due to one of the following: (i) As in the case with DODAB bilayers, each HA$^-$ molecule leaves the gap with a few molecules of STA$^+$ attached to it. This would expose excess hydrophobic chains that would enhance the adhesion force. Since there was no measurable decrease in the bilayer thickness (to within ±3 Å), this effect would be small, and indeed, the adhesion was an order of magnitude smaller than was measured between compressed DODAB bilayers (compare the adhesion force of Figure 6 inset with that of Figure 4b). We note, however, that many previous studies have shown that only a small (a few percent) decrease in the bilayer thickness, or increase in the area per molecule, away from the equilibrium (unstressed) values is sufficient to cause a significant change in the adhesion of bilayers due to the hydrophobic interaction.[12] (ii) An alternative explanation is that some HA chains outside the area of contact bridged the STAB headgroups together via the negative carboxyl groups on HA. This would also cause an enhanced adhesion force.

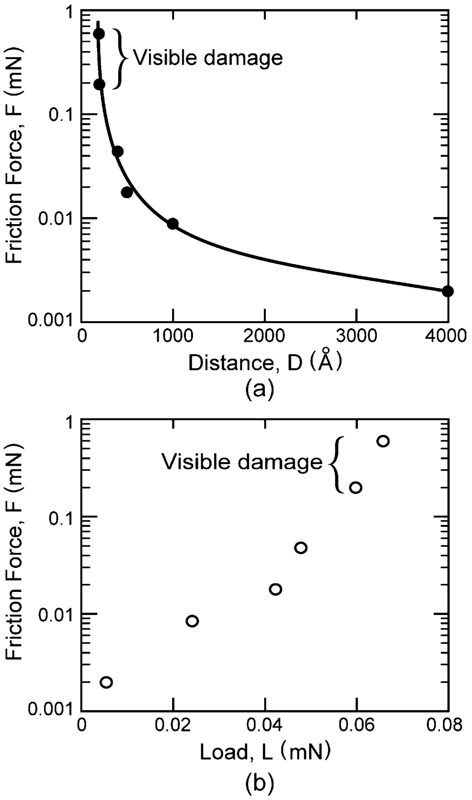
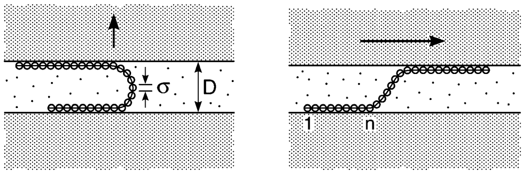


Figure 7. Friction forces at different surface separations between two STAB bilayers measured 10 h after injection of HA into the solution. The shear velocity was kept constant at $v = 0.12$ μm/s and was applied in a saw-tooth (back and forth) motion at a frequency of 0.01 Hz; i.e., the direction of motion was reversed every 50 s. (a) Shear forces vs distance. Once damage is initiated, the friction force increases with sliding time at constant load L, distance D, and sliding velocity v. (b) Friction forces vs load. At low to medium loads, before damage occurs, the coefficient of friction $\mu = F/L$ is roughly constant at 0.35.

Whatever the reason, the fact remains that even under a moderate compressive pressure, all or most of the HA is again squeezed out, and the adhesion between the bilayers is enhanced. This does not augur well for *physisorbed* HA performing as a good boundary lubricant, which will now be described.

3. Shear Lubrication Forces between STAB Bilayers in HA Solutions. The shear (friction) forces of adsorbed HA on STAB surfaces (Figure 7) are very low



(a) (b)

Figure 8. Nonspecific binding of polymer segments to two opposing surfaces: (a) separating surfaces; (b) shearing surfaces.

down to bilayer separations of about $D = 1000$ Å. The friction force increases sharply as D falls below 500 Å, where we expect an increasing number of HA molecules to adsorb on both surfaces (cf. Figure 8b). The low friction at higher separations again suggests that HA can behave as a good lubricant so long as it does not desorb from the gap or bridge the two surfaces.

At higher loads or pressures ($P > 5$ atm), the weakly adsorbed HA was rubbed away from the gap during the shearing, and the surfaces became damaged. This could be readily ascertained from the changing shapes of the optical interference fringes used to continually visualize the surfaces (Figure 3). Thus, both in the above case and in the Ca^{2+} case, HA seems to *initially* protect the mica from damage, which is correlated with the low friction forces down to the point at which damage occurs. Both of these effects may have important implications for the role of HA in synovial fluid, as discussed below.

Damage appears to be caused by one of the following mechanisms: (i) the surfaces come into adhesive contact at some point within the contact zone, which is known to cause surface damage at shearing surface contacts,[57] or (ii) HA bridges form across the gap and give rise to a strong *lateral* adhesion force that results in damage even when the surfaces are still well-separated during shearing. One may ask why bridging produces damage when two surfaces are sheared but not when they are separated.[58] Figure 8 illustrates how, in the special case of high MW polymer-mediated bridging, only a weak *normal* adhesion force is produced (per molecule) when two surfaces are *separated*, but a *strong* lateral force is produced when the surfaces are *sheared*. On separation (Figure 8a), the normal force per molecule is given by $F = \epsilon/\sigma$, where ϵ is the binding energy per segment to the surface and σ is the segment (monomer) length or size. Thus, to move apart a distance σ the force needed is ϵ/σ per polymer molecule. This gives rise to the commonly observed *linear* attractive "bridging" force between two surfaces.[54,59,60] On shearing (Figure 8b), all the bound segments have to move *at the same time* rather than one by one. Thus, to move laterally by distance σ, the force needed is now $n\epsilon/\sigma$ per polymer molecule, where n is the number of segments bound to the surface. For a high-MW polymer such as HA with many binding segments, the value of n can be very large, thereby making the shear forces much larger than the normal forces.[61]

Discussion and Conclusions

HA appears to easily slip between two surfaces and become forced out from the gap between them. Thus, if HA is to function effectively as a boundary lubricant, a stronger force than a mere ionic bond (which is much weaker in water[27]) is required to hold the molecules in the gap. But HA needs more than that, in addition to not *desorbing* from one surface, it must not *adsorb* to the other surface, which increases the friction between them and may cause damage during sliding. Thus, we conjecture that HA could function as a good lubricant so long as it does not desorb from the gap *nor* bridge the two surfaces—conditions that would be satisfied if HA is chemically or specifically bound to each surface. Our experiments also show that the factors that determine whether the friction is low should be distinguished from those that determine whether damage will occur during sliding. Previous work on the lubrication of mica and surfactant bilayer surfaces in water and aqueous salt solutions[62−66] shows that water can be an excellent lubricant, i.e., having a low friction coefficient, but that damage or bilayer fusion can occur at quite low loads or pressures (below 10 atm), especially at sliding velocities above 10 μm/s.

The robustness or fluidity of the surface layers or membranes is also important. HA did not adsorb to the fluid surfaces of DODAB but did to the rigid surfaces of STAB. It is well-known that the forces between fluid surfaces are less adhesive (more repulsive) than between rigid surfaces due to the absence of the repulsive "thermal fluctuation" force between the latter.[27]

When considering the differences between HA and other water-soluble polymers or polyelectrolytes, it is interesting that most other do not generally move away from a gap when compressed or confined between two surfaces.[14,48−56] Instead, they store their elastic energy in a way that can be described by the so-called "elastic" or "storage" modulus component of a viscoelastic system.[67] This normally leads to the so-called "jamming" of confined particles or molecules, which become solid-like or glassy on compression. This is the common scenario for adsorbed polymer layers, especially high-MW polymers.[68] When the compressive load is removed, the polymers reexpand and regain their elastic energy. This also applies to nonadsorbing polymers, especially high-MW polymers at high concentrations, as we have here. And yet this effect appears to be totally absent in the case of adsorbed HA layers where, instead, the molecules are squeezed out with remarkable ease, revealing their "inherent" slipperiness. The very high (negative) charge density on HA, the exposure of many hydrophilic −OH groups per segment, and the great flexibility of the segments—there are six freely rotating bonds per segment as shown in Figure 1c—means (i) that the polymer segments strongly repel each other and therefore want to get away both from themselves and from any negatively charged surfaces (which most biological surfaces are) as much as they can and (ii) that, thanks to its flexibility, it also has the ability to do so. However, for HA to make use of this repulsion and function as an effective lubricant the molecules would have to be *chemically* bound/attached to the surfaces.

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(37) Fringes of equal chromatic order (FECO) which result from the constructive interference of white light between two silver layers across two opposing mica surfaces separated by a small distance and submersed in an aqueous HA solution, together with their corresponding qualitative geometrical images. The geometrical qualitative shapes shown on the left are not in proportion for the sake of clarity—the real thickness of the mica is about a micron, and the separation between the two mica surfaces ranges from a couple of nanometers to 60 nm. The sliding direction of the surfaces is in and out of the paper. The vertical white line on the right is the mercury green calibration line, which happened to coincide very closely with the contact wavelength, corresponding to $D = 0$, of the fringe.

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