Reports

Dynamic Properties of Molecularly Thin Liquid Films

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An experimental technique is described for simultaneously measuring the static and dynamic interactions of very thin liquid films between two surfaces as they are moved normally or laterally relative to each other. Film thickness can be measured and controlled to 1 angstrom. Initial results are presented of the transition in the physical properties of liquid films only one molecular layer thick to thicker films whose properties are practically indistinguishable from the bulk. In particular, the results show that two molecularly smooth surfaces, when close together in simple liquids, slide (shear) past each other while separated by a discrete number of molecular layers, and that the frictional force is "quantized" with the number of layers.

THE PROPERTIES OF SUBMICROSCOPIC quantities of matter, and especially of liquids confined to very small spaces, are of increasing interest, both for a fundamental understanding of molecular systems as well as from a practical need to understand and control many colloidal, interfacial, and material engineering processes. Examples abound, ranging from the formation of small liquid droplets, polymer clusters, or surfactant micelles in solution, to the properties of liquids in very narrow pores or trapped between two solid surfaces (essential to understanding interfacial adhesion, the properties of concentrated dispersions of particles, and lubrication), and to the properties of thin monolayer or bilayer films of surfactants and lipids (the basic structure of biological membranes). Fundamentally, it is important to ascertain how the physical properties of such submicroscopic structures differ from those of the bulk materials, and in particular the size dependence of this variation. Certain bulk, or continuum, properties such as refractive index, dielectric constant, and surface energy appear to be already applicable to individual molecules or to very small clusters of molecules (*1*). In contrast, the results of direct measurements of interaction forces, such as the van der Waals forces between surfaces in liquids, are well described by continuum theories only at large distances, but fail drastically when two surfaces are closer than a few molecular diameters of the intervening liquid (solvent) molecules (*1–5*). This effect is due to the induced (or enhanced) ordering into discrete liquid molecular layers brought about by the approach of the second surface, which leads to a nonuniform variation of the liquid density in such thin films and to an oscillatory force law (*5–7*).

All the above examples refer to equilibrium, or static, properties. Concerning dynamic properties such as viscosity, measurements of the shear viscosities of liquid films down to 8 to 10 molecular diameters thick between two molecularly smooth surfaces show that the first, or at most second, layer of liquid molecules at each surface already exhibits its bulk viscosity (*8, 9*). However, as in the case of the van der Waals interactions, deviations appear to set in for thinner films (*10*). We investigated these deviations (i) to establish whether there are any correlations between the static interactions and dynamic properties of very thin liquid films and (ii) to establish how certain fundamental physical properties vary during the transition from very thin monomolecular films (only one layer thick) to thick liquid films whose properties are practically indistinguishable from the bulk.

The experimental techniques are based on an extension of the surface forces apparatus (*4, 11, 12*) that has previously been used to measure the forces between surfaces in liquids and vapors, wherein two curved mica or mica-coated surfaces are moved normally toward or away from each other and the forces directly measured from the deflection of a force-measuring spring. Figure 1 shows the newly developed lateral sliding mechanism that can be attached to the apparatus and which allows for two surfaces to be sheared past each other at various sliding speeds while simultaneously controlling the normal (compressive or tensile) load and measuring the transverse (frictional) force between them, all these being continuously variable during an experimental run. In addition, the distance between the two surfaces, their shape and exact molecular contact area, and the lateral motion can all be monitored in real time by recording the optical fringe pattern with a video camera and recorder (*8, 10*). This new attachment is similar to one developed by Israelachvili and Tabor (*13*) and used in earlier studies (*13, 14*) of the shear properties of surfactant-coated surfaces in air (that is, exposed to atmospheric conditions). The present device greatly extends the versatility of the technique in the ways described above in addition to having the surfaces in an enclosed chamber where the environment (vapor or liquid) can be controlled.

Sliding was carried out with the initially curved surfaces under various loads that

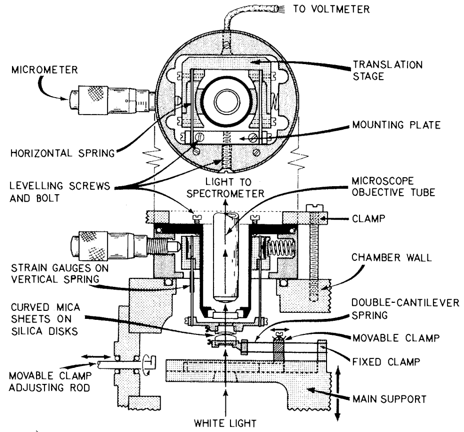

Fig. 1. Lateral sliding mechanism. To enable two surfaces to be slid laterally (transversely) past each other, a new attachment replaced the piezoelectric crystal tube mount supporting the upper silica disk of the basic surface forces apparatus [see figures in (*4, 11, 12*)]. Lateral motion is initiated by a variable speed motor-driven micrometer screw that presses against the translation stage, which is connected through two horizontal double-cantilever strip springs to the rigid mounting plate. The translation stage also supports two vertical double-cantilever springs, which at their lower end are connected to a steel plate supporting the upper silica disk. One of the springs acts as a frictional force detector by having four resistance strain gauges attached to it, which form the four arms of a Wheatstone bridge, and electrically connected through a hole to a sensitive voltmeter or chart recorder. Thus, by rotating the micrometer, the translation stage deflects, causing the upper surface to move horizontally and linearly at a steady rate. If the upper mica surface experiences a transverse frictional or viscous shearing force (due to its contact or proximity to the lower surface), this will cause the vertical springs to deflect, and this deflection can be measured by the strain gauges. The main support, force-measuring double-cantilever spring, movable clamp, white light, and so forth are all parts of the original (unmodified) basic apparatus whose functions are to control the surface separation, vary the externally applied normal load, and measure the separation and normal force between the two surfaces, as previously described in detail (*4, 11, 12*).

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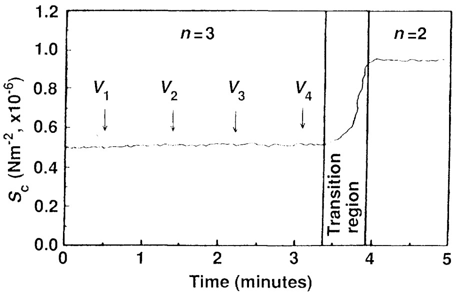

Fig. 2. Sliding of two mica surfaces in cyclohexane, showing the critical shear stress S_c as a function of time ($t = 0$ being an arbitrary time after steady-state sliding was attained) for four different sliding velocities: V_1, V_2, V_3, and V_4 = 0.25, 0.5, 1.0, and 2.0 μm/sec, respectively. In this range of velocities, S_c was independent of velocity so long as the sliding continued with three molecular layers between the surfaces. The increased shear strength observed between 3.5 and 4.0 minutes (at constant velocity and load) coincided with a decrease in the number of molecular layers n between the surfaces from 3 to 2.

caused them to flatten elastically so that sliding occurred between regions where the two surfaces were flat and parallel to each other. Initial results have been obtained for the sliding of two untreated mica surfaces in various liquids, although only those for cyclohexane and octamethylcyclotetrasiloxane (OMCTS) will be described here. The results show that regardless of whether the surfaces are totally immersed in bulk liquid or whether the liquid adsorbs onto the surfaces from the vapor, sliding always occurs with the surfaces separated by a discrete number of molecular liquid layers. Thus sliding occurred at surface separations that were approximately multiples of 5 or 9 Å—corresponding (i) to the mean diameters of the cyclohexane or OMCTS molecules, respectively, and also (ii) to the discrete separations where potential energy minima occur in the equilibrium force laws between mica surfaces across these liquids (*3, 15*). In other words, two surfaces undergoing dynamic shear remain separated by integral layers of liquid molecules corresponding to the same equilibrium separations as occur under static conditions.

A second finding was that the frictional force or shear stress (the ratio of the frictional force to the contact area) depended critically on the number of layers and could not be accounted for in terms of a continuum model where the liquid film is assumed to have bulk Newtonian viscosity. For example, with one, two, or three molecular layers between the surfaces, a certain "critical" shear stress S_c must be attained to initiate sliding, after which the surfaces move at a constant S_c independent of sliding velocity or applied load (compressive or tensile) so long as sliding continues with the same number of boundary layers between the surfaces (although this number is dependent on the velocity and load). Typical results are given in Fig. 2. None of this behavior can be explained in terms of a purely Newtonian viscous fluid film (as in plane Couette flow) nor in terms of conventional frictional sliding. Moreover, the value of S_c was much higher than expected for any purely viscous flow. For example, in cyclohexane, $S_c = 2 \times 10^7$ Nm^{-2} when sliding occurs at velocities of 0.25 to 2 μm/sec with only one layer of boundary molecules (5 Å thick), a value that is about seven orders of magnitude higher than expected for purely viscous flow.

The one important factor on which the critical shear stress depended was the number of boundary liquid layers (Table 1); in cyclohexane, for example, S_c fell by about an order of magnitude per additional layer. In other words, the friction is "quantized" depending on the number of molecular layers separating the surfaces. By extrapolation one may infer that when seven to ten layers are present, the shear stress of the liquid film would have fallen to the value expected for bulk continuum Newtonian flow. It is noteworthy that this is about the same number of layers when the forces across a thin fluid film begin to be describable by continuum theories (*1–4*). Many more experiments will have to be carried out, especially with other liquids and surfaces, before a comprehensive picture emerges.

There are many conclusions that may be drawn from the results described above. First, there is a direct correspondence between the potential minima characteristic of the equilibrium oscillatory force laws between surfaces (*1, 3, 15*) and the discrete number of layers at which two surfaces slide past each other. In both cases, deviations from continuum behavior are observed as the number of molecular layers falls from about ten to one, where the mechanism of shear flow and the whole concept of "viscosity" become qualitatively different from that occurring with thick films. When these results are compared with previous measurements of the viscosity in thin liquid films (8 to 10 molecular diameters thick), a number of other important implications emerge, including the conclusion that the position of the nonslip plane, and hence the structure and mobility of liquid molecules, at one surface must be a function of its distance from a second surface. Qualitatively, our results are in agreement with some recent molecular dynamics simulations of Lennard-Jones liquids in micropores (*16*), which predict layered flow, with deviations from bulk behavior becoming significant in pores less than 10 molecular diameters.

The results presented here, especially the discrete (quantized) nature of certain interfacial processes, expose a new and previously unsuspected picture of the mechanism of dynamic interactions occurring at the molecular level. They offer new fundamental insights into the interactions of liquids with surfaces and for liquids confined or flowing within very thin films, cracks, or small pores. They also have an obvious bearing on many practical applications in the fields of colloidal particle interactions, the properties of grain boundary phases of composite materials such as ceramics (*17*), flow in thin liquid films and lipid bilayers, rheology, adhesion, lubrication, and tribology.

Table 1. Critical shear stress S_c for integral number of liquid layers n trapped between two mica surfaces for cyclohexane (molecular diameter ≈5 Å) and octamethylcyclotetrasiloxane [OMTCS, molecular diameter ≈8.5 Å (*2, 3*)]. The significantly lower value of S_c for one layer of OMCTS (compared to cyclohexane) is presumably due to its larger size; to the larger OMCTS molecules the mica surfaces appear "smoother" (on a molecular scale) and thereby allow them to roll more easily between the two surfaces. The results represent an average of five multiple runs in a single experiment.

n	S_c (N/m^2)	
	OMCTS	Cyclohexane
1	$8.0 \pm 0.5 \times 10^6$	$2.3 \pm 0.6 \times 10^7$
2	$6.0 \pm 1.0 \times 10^6$	$1.0 \pm 0.2 \times 10^6$
3	$3.0 \pm 1.0 \times 10^6$	$4.3 \pm 1.5 \times 10^5$
4	Not measured	$2.0 \pm 1.0 \times 10^4$

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Tertiary Structure Is a Principal Determinant to Protein Deamidation

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The protein deamidation process involves the conversion of the amide side-chain moieties of asparagine and glutamine residues to carboxyl groups. This conversion is an unusual form of protein modification in that it requires catalysis by an intramolecular reaction where both the substrate (asparagine and glutamine side chains) and "catalytic site" (the peptide nitrogen of the succeeding residue) are constituents of several consecutive residues along the polypeptide chain. The stereochemical factors governing this process were studied with a data base derived from the neutron crystallographic structure of trypsin from which amide groups and oxygen can be unambiguously differentiated because of their different neutron scattering properties. The neutron structure allowed for the direct determination of those residues that were deamidated; 3 of 13 asparagine residues were found to be modified. These modified residues were clearly distinguished by a distinct local conformation and hydrogen-bonding structure in contrast to those observed for the other asparagine residues. No correlation was found between preference to deamidate and the chemical character of residues flanking the site, as had been proposed from previous peptide studies.

DEAMIDATION OF ASPARAGINE RESIDUES is a commonly observed form of post-translational protein modification (*1–3*). The process may play an essential role in the degradation and clearance of proteins and may be under genetic control through sequence variation to adjust rates of degradation (*4–6*). The generally accepted mechanism of deamidation involves the formation of an intramolecular cyclic imide intermediate that can break down to replace the amide substituent group with a carboxyl group. The breakdown can proceed by either of two distinct pathways (Fig. 1) to form either a normal peptide linkage (that is, Asn to Asp) or a β-carboxyl linkage (*7*). Peptide studies have shown that glutamine can also undergo deamidation, but at much reduced rates (*2*).

Studies attempting to identify the stereochemical factors affecting deamidation have centered on the use of synthetic peptides and focused on the role that particular sets of flanking side-chain types (that is, X-Asn-Y) have on the process (*1, 4*). However, in the native folded state of the protein, tertiary structure would be expected to affect significantly the susceptability of amide side chains to undergo modification. In this study structural features were identified that affected the deamidation process and an attempt has been made to determine the relative importance of sequence versus conformational factors in promoting this type of intramolecular modification. Trypsin represents an excellent system to study because the sequences and conformations of the adjacent segments of polypeptide chain containing the asparagine residues show significant diversity.

Detailed studies of the interrelation between structural factors and deamidation have been limited because of the difficulties involved in identifying modified groups. Neutron diffraction, by virtue of its ability to observe hydrogen and deuterium atoms in large biomolecules, is ideally suited to identify deamidated residues in proteins. The interpretation of the state of amidation of Asn side chains in trypsin (*8*) was drawn from data taken from two highly refined neutron structures: a D_2O structure (the crystal was soaked in D_2O to exchange waters of crystallization and labile protons), $R = 0.190$ at 1.8 Å resolution, and a H_2O structure, $R = 0.197$ at 2.1 Å resolution (*9*). In a D_2O structure, the large scattering differences between oxygen (5.8 fermi) (*10*) compared with a nitrogen and two deuteriums (22.2 fermi) make the assignment of the orientation of amide side chains unambiguous (*9*). In the D_2O-trypsin analysis, 10 of the 13 well-ordered amide side chains refined to give reasonable temperature factor values (*11*) for all atoms. However, for Asn^{48}, Asn^{95}, and Asn^{115}, the temperature factors for the two side-chain deuterium atoms were greater than 80 Å^2 (these atoms have essentially no scattering contribution) compared with an average of about 15 Å^2 for the other side-chain atoms in these groups. A comparison of the structure around these asparagines to other asparagines and other side-chain groups with bonded deuteriums showed that the phasing model was of sufficient quality to have observed the deuteriums on the three Asp(Asn) side chains if they were present.

To further confirm these findings, the D_2O and H_2O structures were combined to calculate a D_2O-H_2O difference Fourier map, which is an extremely sensitive method for locating deuterium atoms (*12*). Because of the substantial scattering differences between hydrogen (−3.8 fermi) and deuterium (+6.6 fermi), amide groups produce large peaks in these difference maps; these maps are also less prone to phasing bias and effects due to partial exchange and disorder (*12*). Significant density was observed for the amide deuterium sites except for Asn^{48}, Asn^{95}, and Asn^{115}, which were nearly featureless (Fig. 2) (*13*). This result indicated that there were no exchangeable groups associated with these three side chains. This observation, together with the D_2O refinement results, offered extremely strong support for the interpretation that these asparagines had been deamidated.

An analysis of the conformational pattern and hydrogen-bonding interactions localized around Asn residues (Table 1) suggests

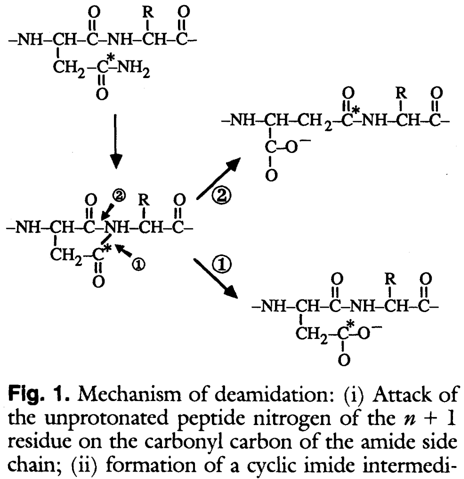

Fig. 1. Mechanism of deamidation: (i) Attack of the unprotonated peptide nitrogen of the $n + 1$ residue on the carbonyl carbon of the amide side chain; (ii) formation of a cyclic imide intermediate; (iii) attack by water at position 1 or 2 leading to a breakdown of the intermediate; (iv) formation of either the NH_2 to O substitution or the β-carboxyl insertion. The asterisk marks the position of the side-chain carbonyl carbon. In the β-carboxyl linkage, this carbon becomes part of the main chain and increases the chain length by one atom.

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