Hydrophobic Forces and Hydrogen Bonds in the Adhesion between Retinoid-Coated Surfaces

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Interactions between hydrophobic chains of lipid monolayers and interactions between hydrophilic headgroups of lipid bilayers (with or without a molecular recognition step) are now well documented, especially for commonly used lipids. Here, we report force measurements between a new class of fluorinated lipid layers whose headgroups (synthetic ligands of retinoid receptors) display a very unusual polar/apolar character and can interact via a combination of hydrophobic forces and hydrogen bonds. Although these two interactions produce adhesion and are therefore not easily distinguishable, we show that it is possible to extract both contributions unambiguously. Experiments are performed both in pure water, where the adhesion is a combination of hydrophobic forces and hydrogen bonds, and in Tris buffer, where the hydrophobic effect is the dominant short-range attractive force. The contribution of hydrophobic forces scaled down to molecular interactions is deduced from force versus distance profiles, and the same value is found independently in pure water and Tris buffer, about 1 k_BT. We also show that retinoid lipid layers attract each other through a very long-range (100 nm) exponential force, which is insensitive to the pH and the salinity. The origin of this long-range attraction is discussed on the basis of previously proposed mechanisms.

Introduction

Hydrophobic forces and hydrogen bonds are weak interactions, which are involved in many biochemical processes, including enzyme−substrate, antibody−antigen, protein−DNA, and receptor−ligand interactions. They also both contribute to the stability of macromolecular assemblies, such as the three-dimensional structures of proteins and nucleic acids, and play a central role in the protein folding process. The mechanism that leads from a random coil of amino-acids to the folded conformation state of a functional protein is still poorly understood. In the existing models, a hydrophobic collapse either precedes or follows the formation of hydrogen-bonding secondary structures.[1,2] Recent investigations even suggest that both processes are tightly linked and that the presence of hydrophobic residues in the vicinity of hydrogen-bonding groups enhances the stability of the latter by creating a dehydrated environment, which protects them from water attack.[3,4] The existence of such a combined effect of hydrophobic forces and hydrogen bonds is still disputed, and its stabilization energy is unknown. The way by which nature uses weak interactions, such as hydrophobic forces and hydrogen bonds, has also been a source of inspiration, in supramolecular chemistry, for the development of materials with novel properties.[5−7] For instance, organic thin films with unique electro-optical properties can be generated by using the capacity of certain aromatic compounds to form favorable hydrophobic, π-stacking, and hydrogen-bonding interactions.[8] Therefore, the ability to quantify the combined effect of hydrophobic forces and hydrogen bonds is fundamentally important across various scientific fields. The Surface Force Apparatus (SFA[9]), which allows the measurement of force versus distance between two surfaces, is ideally suited for conducting such a measurement. Its high resolution in distance and force has already allowed a careful experimental characterization of several fundamental and specific interaction forces.[10−13] Some long-range attractive forces have been measured between many different surfaces, including purely hydrophobic surfaces[14,15] as well as certain hydrogen-bonding surfaces,[12,16,17] but their mechanism still remains controversial, and none of the proposed models can explain all of the experimental observations.[18,19] On the other hand, many short-range attractive forces (D < 10 nm) have been well characterized through the adhesion of functionalized lipid layers (e.g., hydrogen-bonding or chelating lipid layers[13,17]), and the corresponding molecular binding energies were measured accurately. In a situation where several short-range attractive forces act concomitantly, it remains however a real challenge to determine the pure contribution of each of them unambiguously. In the present study, force measurements are performed between two model surfaces, which display both hydrophobic and hydrogen-bonding sites. The surfaces are bilayers of a retinoid lipid whose headgroup comprises two

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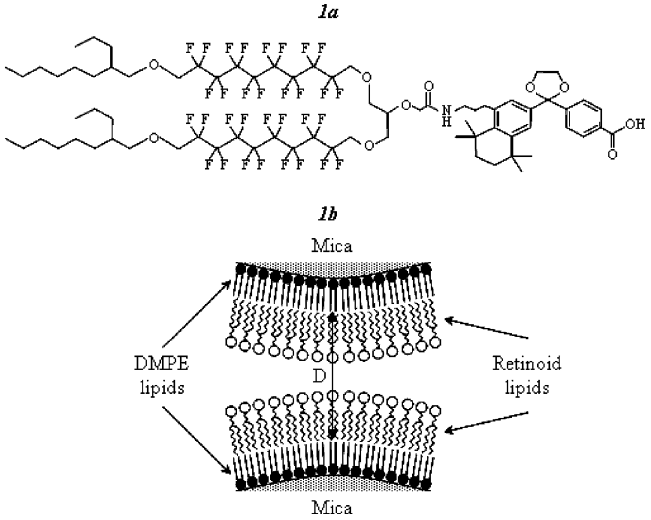

Figure 1. (a) Structure of the retinoid lipid. The headgroup contains two aromatic rings (benzene and tetrahydronaphthalene), which are highly hydrophobic, and one carboxyl group, which can establish hydrogen bonds in water. It is connected to two ramified and partially fluorinated hydrophobic tails through a flexible spacer. Because hydrocarbon and fluorocarbon compounds are not miscible, the retinoid headgroup remains accessible in solution. (b) Bilayers used in the course of SFA experiments. The inner monolayers are made of DMPE lipids to render the mica hydrophobic, and the outer monolayers are made of retinoid lipids. The reference distance ($D = 0$) is the distance between the hydrophobic sides of the DMPE monolayers.

aromatic rings (a benzene ring and a tetrahydronaphthalene ring), which are highly hydrophobic, and one carboxyl group, which can establish hydrogen bonds in water (Figure 1a). The hydrogen-bonding sites, and therefore their effect on the interaction forces, can be suppressed by increasing the pH of the electrolyte. This allows us to determine the respective contributions of hydrophobic forces and hydrogen bonds in a situation where they both contribute to the adhesion. Force measurements are therefore conducted both in pure water (pH $\approx$ 5.5) and in 10 mM Tris buffer (pH 8.0). The resulting interaction profiles provide information on long-range attraction between two retinoid surfaces and allow the quantification of short-range hydrophobic forces at the molecular scale.

Materials and Methods

Chemicals. Dimyristoyl-phosphatidyl-ethanolamine (DMPE) lipid was purchased from Avanti Polar Lipids as 10 mg/mL chloroform solution. Tris(hydroxymethyl)aminomethane (Tris) was obtained from Merck-Eurolab as powder and was solubilized at 10 mM in ultrapure water (purified with the Elgastat Maxima system, HPLC model). Diluted HCl was used to adjust the pH to 8.0.

A synthetic ligand of the retinoic acid receptor RXR, 4-[2-(5,6,7,8-tetramethyl-2-naphthalenyl)-1,3-dioxolan-2-yl] benzoic acid[20] (Figure 1a), was used here as a model system displaying both hydrophobic and hydrogen-bonding sites. It was chemically coupled to the hydrophobic moiety of a partially fluorinated lipid via a flexible spacer as described previously;[21] this synthetic lipid will be called retinoid lipid in the rest of this Article. Two ramifications introduced in the hydrophobic chain ensure that the lipid is in a fluid state and thus allow the functional groups to have a translational freedom within the monolayers. The non-miscibility between hydrocarbons and fluorocarbons, combined with the use of a sufficiently short hydrophilic spacer, ensures the accessibility and the rotational freedom of the functional groups in solution.[22] Therefore, when incorporated in a lipid monolayer, the functional groups can adopt the most favorable configuration to interact with their cognate residing in the opposite monolayer.

Surface Force Apparatus (SFA). Force measurements between lipid bilayers are performed with the Surface Force Apparatus (SFA[9]) technique, which gives the force F between two surfaces arranged in a crossed-cylinders geometry (radius R) as a function of their separation distance D. The force ($\pm 1\ \mu$N) is given by the deflection of a bending spring connected to one of the two surfaces. The distance (± 2 Å) is obtained interferometrically, by using two silver-coated surfaces. The ratio $F(D)/R$ is proportional to the interaction free energy $E(D)$ per unit area between two plane surfaces:

$$\frac{F(D)}{R} = 2\pi E(D) \qquad (1)$$

Lipid bilayers are formed on silvered mica surfaces by the Langmuir−Blodgett deposition technique. The inner monolayer (with its headgroups facing the mica surface) is composed of DMPE lipids, deposited in a solid state at 38 mN/m. The outer monolayer (with its headgroups facing the aqueous medium) is composed of retinoid lipids, deposited in a fluid state either at 30 mN/m (experiments in pure water) or at 35 mN/m (experiments in Tris buffer). The reference distance $D = 0$ of SFA curves corresponds to the contact between DMPE hydrophobic tails (Figure 1b). It is determined at the end of a force experiment through a DMPE/DMPE contact in air (after removal of the aqueous buffer and therefore of the two outer monolayers). Force measurements consist of cycles of approaching and then separating the surfaces. To probe for the reproducibility of the interaction forces, at least two different experiments, that is, with different pairs of bilayers, are performed, and, in each experiment, three different approaching−separation cycles are realized. The standard deviation of the measured adhesion free energy per unit area is 3 mN/m (Table 1).

Results and Discussion

Retinoid Monolayer Isotherm and Stability. Monolayers of the retinoid lipid were formed at the air/water or at the air/Tris interface, and their surface pressure versus molecular area isotherms were recorded (Figure 2).

In both cases, the lipid forms fluid monolayers, even at high surface pressure, as expected from the presence of the branched alkyl chains in its hydrophobic tail. Monolayers are more compressible on a water subphase than on a Tris subphase. This is due to electrostatic repulsions that take place, in Tris buffer, between ionized carboxyl groups. In both cases, the collapse of the monolayer is reached for a molecular area of 1.05 nm^2, but the corresponding surface pressure is lower for monolayers formed on pure water (31 mN/m on pure water versus 44 mN/m on Tris buffer). This phase transition from two to three dimensions can be completely reversed on a water subphase: the isotherm measured during expansion is strictly identical to the one measured upon compression. In contrast, on a Tris subphase, the isotherms measured during both expansion and recompression of the monolayer show a significant hysteresis (data not shown). This more cohesive behavior of the monolayer on water is most likely due to the formation of folds toward the air, which are stabilized by hydrogen bonds between the retinoid headgroups. Such folds cannot form when the retinoid monolayer is spread on a Tris buffer, because most of the carboxyl groups are ionized and thus unable to form any hydrogen bond. In that case, when the monolayer is compressed above the collapse pressure, some lipids irreversibly leave the monolayer structure.

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Table 1. Parameters of the Interaction between Two Retinoid Lipid Layers in Pure Water and in Tris Buffer (for Definitions, See the Text)

	F_0/R (mN/m)	F_e/R (mN/m)	F_{0eff}/R (mN/m)	E_{0eff} (mJ/m^2)	σ_R (nm^2)	w_{H-bond}[17] (k_BT)	$w_{hydrophobic}$ (k_BT)
water pH $\approx$ 5.5	38 $\pm$ 3	0	38 $\pm$ 3	8.2 $\pm$ 0.6	1.09	1.5	0.7 $\pm$ 0.1
Tris pH 8.0	8 $\pm$ 3	7 $\pm$ 2	15 $\pm$ 4	3.2 $\pm$ 0.9	1.19	0	0.9 $\pm$ 0.3

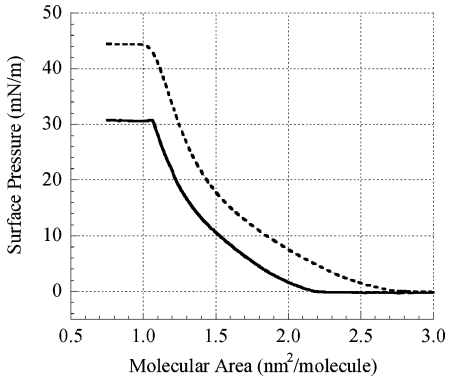

Figure 2. Compression isotherms of retinoid monolayers when deposited at the air/water (pH $\approx$ 5.5, solid line) or at the air/Tris (pH = 8.0, dashed line) interface. In both cases, the monolayer is in a fluid state, even at high surface pressure. Retinoid monolayers are more compressible on water, where there is less electrostatic repulsion between the carboxyl groups. The collapse pressure is lower on water (31 mN/m) than on Tris buffer (44 mN/m). In both cases, the molecular area at collapse is 1.05 nm^2.

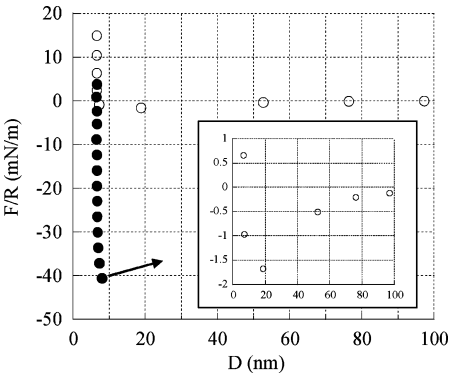

Figure 3. Interaction profile of two retinoid monolayers in pure water. Approaching data are represented by ○ and separation data by ●. The two surfaces attract each other at long distance (100 nm) and jump into adhesive contact from a distance of about 20 nm (see inset). The resulting distance, 6 nm, corresponds to two compact retinoid monolayers in contact. The pull off force necessary to separate two contacting retinoid monolayers (F_0/R) is 38 $\pm$ 3 mN/m (average over several independent SFA experiments). The corresponding jump out of contact is symbolized by the arrow.

The stability of the retinoid monolayer relative to desorption was checked at the air/water (π = 30 mN/m), air/Tris (π = 35 mN/m), mica/water, and mica/Tris interfaces, essentially as described previously.[17] In all cases, it was very stable, with an average desorption of less than 0.5% per hour. This ensures that the retinoid lipid forms stable close-packed monolayers on the time scale of force measurement experiments (about 6 h).

Hydrogen-Bonding and Hydrophobic Forces between Retinoid Lipid Layers in Pure Water. SFA experiments were first conducted in pure water between two retinoid monolayers deposited on hydrophobized mica surfaces. A representative force versus distance profile is displayed in Figure 3.

Between 200 and 100 nm, no force is detected between the two monolayers. A long-range attractive force sets in around 100 nm (inset in Figure 3). The characteristics and the origin of this attraction will be discussed further below. When the gradient of the attractive force is equal to the spring stiffness, the surfaces jump into an adhesive contact and flatten (data not shown). As is often observed with lipids, a sharp repulsion then takes place when the force is further increased (typically up to about 10 mN/m). Upon separating the surfaces, an adhesion is felt and the separation distance remains constant. When the pulling force is equal to the force that binds the two monolayers, the surfaces jump out of contact (adhesive jump). This pull off force F_0 is proportional to the adhesion free energy per unit area E_0 between the two monolayers. The relation between F_0 and E_0 depends on the elasticity of the two surfaces relative to the strength of their adhesion force.[23,24] Here, because the contact zone between the two surfaces is flat, the appropriate theory is the JKR one:[25]

$$\frac{F_0}{R} = \frac{3\pi E_0}{2} \tag{2}$$

We measure F_0/R = 38 $\pm$ 3 mN/m, which gives E_0 = 8.2 $\pm$ 0.6 mJ/m^2. This is much stronger than the adhesion energies usually observed between classical phospholipid monolayers[26] (between 0.1 and 0.8 mJ/m^2). This suggests that two retinoid monolayers adhere via some specific interactions. The retinoid lipid headgroup contains a free carboxyl group, which can establish hydrogen bonds, and some aromatic rings, which are highly hydrophobic. The measured adhesion free energy per unit area can thus be written as a combination of hydrogen-bonding and hydrophobic interactions:

$$E_0 = \frac{1}{\sigma_R}(w_{H-bond} + w_{hydrophobic}) \tag{3}$$

where w_{H-bond} and $w_{hydrophobic}$ are, respectively, the average contribution of hydrogen-bonding and hydrophobic forces to the adhesion free energy per headgroup area; σ_R is the area occupied by a retinoid lipid within its monolayer. This area can be deduced from the retinoid monolayer compression isotherm on pure water (Figure 2): σ_R = 1.09 nm^2.

To evaluate the contribution of hydrogen bonds to the measured adhesion force, one first needs to know the ionization state of the carboxyl groups at the monolayer surface. The approaching phase of the force versus distance profile displays no electrostatic double layer repulsion (the Debye screening length of water is expected to be of 170 nm at pH 5.5). Because the SFA technique allows the detection of less than 0.1% charge within two interacting monolayers,[27] this ensures that most of the carboxyl groups are protonated in pure water. Hydrogen-bond energies have been thoroughly characterized in previous studies involving nucleoside and nitrilotriacetate (NTA) lipid layers;[17] their

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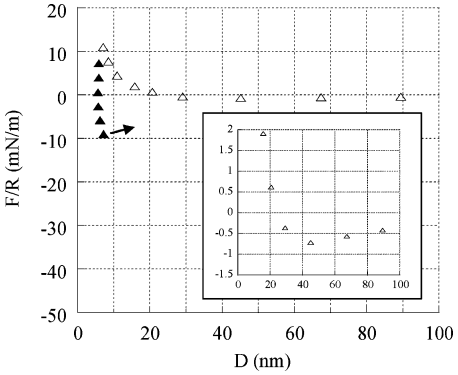

Figure 4. Interaction profile of two retinoid monolayers in 10 mM Tris buffer. Approaching data are represented by △ and separation data by ▲. As in water, the two surfaces attract each other from 100 nm. The electrostatic double-layer repulsion between charged retinoid monolayers sets in around 30 nm and is superposed on this long-range attractive force (see inset). The pull off force for two contacting retinoid monolayers (F_0/R) is 8 ± 3 mN/m (average over several independent SFA experiments), which is much smaller than that in pure water. The corresponding adhesive jump is symbolized by the arrow.

contribution to the adhesion free energy per headgroup area is:

$$w_{\text{H-bond}} = \frac{2e_{\text{H}}}{1 + \exp(-e_{\text{H}})} \tag{4}$$

where the factor 2 comes from the two hydrogen bonds that can form between each pair of carboxyl groups, and e_{H} is the energy of a single hydrogen bond in pure water ($e_{\text{H}} = 1\ k_{\text{B}}T$[17]).

The contribution of hydrophobic forces is then directly deduced from eq 3; this gives:

$$w_{\text{hydrophobic}} = 0.7 \pm 0.1 k_{\text{B}}T$$

All parameters relevant for the interaction between two retinoid layers in pure water are summarized in Table 1.

Electrostatic and Hydrophobic Forces between Retinoid Lipid Layers in Tris Buffer. Force measurements between retinoid monolayers were next conducted in 10 mM Tris buffer, pH 8.0. The corresponding force versus distance profile is displayed in Figure 4. As in pure water, a long-range attractive regime sets in at 100 nm. Around 30 nm, a repulsive force is superposed on the long-range attractive force (inset in Figure 4). This force corresponds to the electrostatic double-layer repulsion between two charged retinoid surfaces and can be fitted by a 5 nm decay length exponential curve. This is in good agreement with the expected 4 nm Debye screening length of a 10 mM Tris electrolyte. No sudden inward jump is observed in Tris buffer. Instead, the surfaces can be progressively approached and the repulsive force slowly brought to about 10 mN/m. The resulting distance is about 6 nm, which corresponds to the thickness of two close-packed retinoid monolayers in contact. An adhesive jump is observed when separating the two surfaces, but the corresponding pull off force is substantially smaller than that in pure water: $F_0/R = 8 \pm 3$ mN/m. This is not surprising because the electrostatic double-layer force facilitates the separation of two contacting monolayers and therefore reduces the pull off force.[13,26] Its contribution thus has to be taken into account to compare pull off forces measured in water to the ones measured in Tris buffer. It is appropriate to consider the effective pull off force, $F_{0\text{eff}}/R$, defined as the pull off force that would be measured in the absence of any electrostatic double-layer repulsion, F_e/R ($F_{0\text{eff}}/R$ thus gives the pure contribution of the adhesive forces to the measured pull off force):

$$\frac{F_{0\text{eff}}}{R} = \frac{F_0}{R} + \frac{F_e}{R} \tag{5}$$

where F_e/R is evaluated at the distance of the adhesive jump (Table 1). This gives $F_{0\text{eff}}/R = 15 \pm 4$ mN/m.

What is the contribution of hydrogen bonding to this effective pull off force? In the present case, ammonium cations $(HOCH_2)_3CNH_3^+$ brought by the dissociation of Tris-HCl outnumber cations H^+ by at least 5 orders of magnitude. Therefore, each time a carboxyl group is ionized, its H^+ counterion is replaced by $(HOCH_2)_3CNH_3^+$, which then prevents the formation of any hydrogen bond. At equilibrium, all hydrogen-bonding sites are occupied, and the only contribution remaining is the one due to the hydrophobic effect. The contribution of hydrophobic forces to the adhesion free energy per headgroup area can thus be written as:

$$w_{\text{hydrophobic}} = \sigma_{\text{R}} \frac{2F_{0\text{eff}}}{3\pi R} \tag{6}$$

with $\sigma_{\text{R}} = 1.19$ nm^2 (Figure 2), we obtain:

$$w_{\text{hydrophobic}} = 0.9 \pm 0.3 k_{\text{B}}T$$

All parameters relevant for the interaction between two retinoid layers in Tris buffer are summarized in Table 1.

The hydrophobic contribution extrapolated to molecular interactions is therefore the same in pure water and in Tris buffer, about 1 $k_{\text{B}}T$. Because the two values were calculated independently, this validates the quantitative analysis and suggests that (i) hydrogen-bonding and hydrophobic interactions can be, to a good approximation, considered as additive, and (ii) hydrophobic forces are mostly insensitive to the pH and the salinity of the buffer. This value is about 3 times weaker than what is generally observed between hydrophobized mica surfaces.[18,28] It is reasonable because, within the retinoid headgroups, the hydrophobic compounds, benzene and tetrahydronaphthalene, are interweaved with some hydrophilic carboxyl groups (Figure 1a), which is sufficient to ensure an amphiphilic character and therefore reduce the hydrophobicity.

Long-Range Attractive Force between Retinoid Lipid Layers. In pure water and in Tris buffer, two retinoid lipid monolayers attract each other through a long-range exponential force, which is detected at around 100 nm and presents a decay length of about 40 nm (Figure 5). This exponential attraction extrapolated to the contact distance of two retinoid layers gives a force much weaker than the measured pull off forces. This suggests that the long-range attraction and the adhesion are governed by two different mechanisms. Because the long-range attractive force persists, and is of the same range, in Tris buffer, where the Debye screening length is of 4 nm, one can conclude that it cannot have a conventional electrostatic origin as in a recent study on charge-mosaic surfaces.[29] In addition, because there are no more hydrogen bonds in Tris buffer, one can deduce that the observed attraction is not likely related to hydrogen bonding but rather comes from the hydrophobic parts of the molecules.

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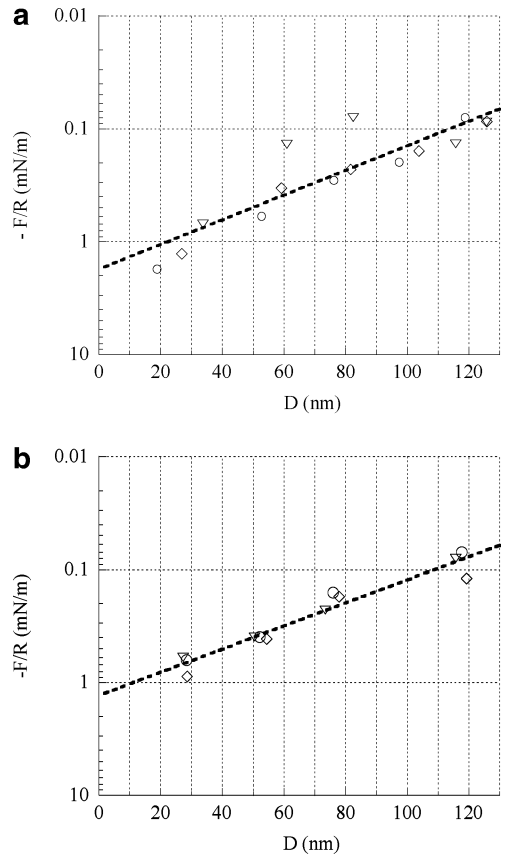

Figure 5. Closer view of the long-range attractive force between two retinoid monolayers in pure water (a) and in Tris buffer (b). Data are displayed on a semilog scale. Each symbol represents a different SFA experiment. For data in Tris buffer, the contribution of the electrostatic double-layer repulsion has been first subtracted to the force curves. These attractive forces can be fitted with a simple exponential function having a decay length of 40 nm (dashed lines).

Similar long-range attractive forces have already been observed between two hydrophobic monolayers,[14,15] one hydrophilic and one hydrophobic surface,[19,30,31] two mixed monolayers (presenting both a hydrophobic and a hydrophilic behavior),[32] two charge-mosaic surfaces,[29] and other hydrogen-bonding surfaces (nucleosides and NTA).[12,16,17] The origin of this attraction still remains unknown, and none of the proposed theories account for all of the experimental observations; they include metastability of the water film between the two surfaces,[33,34] correlated dipole−dipole interactions,[15,35] or water structural effects.[36,37]

The common point between retinoid surfaces and previously studied nucleoside and NTA surfaces is the potential ability of the lipids to form ordered domains within their monolayer. In fact, it has already been demonstrated that nucleoside lipid monolayers present a translational order arising from π-stacking interactions between the headgroups.[38] The same type of interaction may exist as well between the aromatic rings of the retinoid headgroups. In the case of NTA lipid monolayers, one or more of the three carboxyl functions of each NTA group could be involved in intra-layer hydrogen bonds, which would lead to the formation of similar ordered domains. Such domains would generate some dipole−dipole forces between the two interacting surfaces, whose distance range is of the same order as the domain size.[15]

Surprisingly, this long-range attractive force is the same both in pure water (pH $\approx$ 5.5) and in 10 mM Tris buffer (pH 8.0), which suggests that it is insensitive to the salinity and to the pH. A persistence of long-range attractions in the presence of moderate amounts of electrolyte has already been observed in previous works.[37,39] Moreover, one must keep in mind that the electrolyte screens the electrical field but also reduces lateral repulsions between lipid molecules and can therefore lead to the formation of larger domains. By increasing the domains size, it increases the range of dipole−dipole interactions and partly compensates for the effect of screening.

Conclusion

Surface force measurements made in the past with lipid layers involved mostly interactions between hydrophilic heads of bilayers (bearing or not specific binding groups) or interactions between hydrophobic tails of monolayers. Here, we have used a lipid whose headgroup displays a very unusual polar/apolar character and can form hydrogen bonds through its carboxyl function and, at the same time, establish hydrophobic interaction through its aromatic rings. By controlling the pH of the working buffer, we were able to obtain situations where the interaction was either dominated by hydrogen-bonding and hydrophobic forces (pure water, pH $\approx$ 5.5) or by electrostatic double-layer forces and hydrophobic forces (Tris buffer, pH 8.0). In pure water, all carboxyl groups were protonated and could therefore establish hydrogen bonds. In Tris buffer, all hydrogen-bonding sites were occupied by ammonium cations resulting from the dissociation of Tris-HCl, and the adhesion energy decreased by a factor of almost 5. This extreme sensitivity to the pH is due to the combination of two effects: (i) the ionization of carboxyl groups, which generates some electrostatic double-layer repulsion, and (ii) the subsequent vanishing of the hydrogen bonds. The force data allowed the estimation of the strength of the hydrophobic interaction at the scale of one molecule, about 1 k_BT, a value that is independent of the pH and the salinity of the buffer. Our force versus distance profiles also showed that two retinoid monolayers attract each other through a very long-range exponential force, whose exact mechanism remains to be established.

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