Long-Range Attraction between Nucleosides with Short-Range Specificity: Direct Measurements

Frédéric Pincet,[1] Eric Perez,[1] Gary Bryant,[1] Luc Lebeau,[2] and Charles Mioskowski[2]
[1] *Laboratoire de Physique Statistique de l'Ecole Normale Supérieure, associé aux universités Paris VI et Paris VII, 24, rue Lhomond, 75231 Paris Cedex 05, France*
[2] *Laboratoire de Synthèse Bio-organique, CNRS, Unité de Recherche Associée 1386, Faculté de Pharmacie, 74 Route du Rhin, 67401 Illkirch, France*

(Received 28 March 1994)

The structure of DNA is the result of highly specific interactions between nucleotides (adenine and thymine, cytosine and guanine) based on hydrogen bonds and size complementarities. We performed direct measurements of the forces between adenosine and thymidine using a surface force apparatus [J. Chem. Soc. Faraday I **74**, 975–1001 (1978)]. These measurements showed that without the size effect hydrogen bonds alone generate the specificity. Bond energies obtained in our experiments are consistent with estimates indirectly obtained through other methods. We have also observed an unexpected long-range nonspecific attraction.

PACS numbers: 87.15.Kg, 68.18.+p, 68.35.Md, 87.15.By

Specific interactions occur extensively in living matter and are presumably due to known physicochemical interactions together with geometrical constraints. However, their ranges and the ways by which different effects combine to produce specific interactions are largely unknown. The availability of new techniques which can be used to directly investigate force-distance relationships among biomolecules now make these interactions accessible. Only one key-lock interaction has been directly measured, that between streptavidin and biotin [1,2], displaying an 8.5 nm range specific attraction. One of the most common and simple specific interactions is that between nucleotides [3]. The distance between the two DNA strands is constant and the size of thymine is complementary to that of adenine relative to this distance; this size effect, combined with hydrogen bonds, produces the preferential interaction. To date, the energy of specific bonds between complementary bases has been obtained only from thermodynamic studies or quantum mechanic calculations [4,5].

Here, we present direct force measurements between nucleosides performed while controlling the orientation and accessibility of the functions of the molecules and without the geometrical constraints present in DNA. The aim was to obtain the binding energies, the range of the specific interaction, and the importance of hydrogen bonding relative to the geometric effects. For the purpose of this study, we synthesized two lipids (see Fig. 1), DOPHS-5′T and DOPHS-5′A, which have thymidine and deoxyadenosine, respectively, as the headgroups. These lipids could be deposited [6], by the Langmuir-Blodgett technique, onto mica surfaces and the forces between such surfaces measured with a surface force apparatus (SFA) [7].

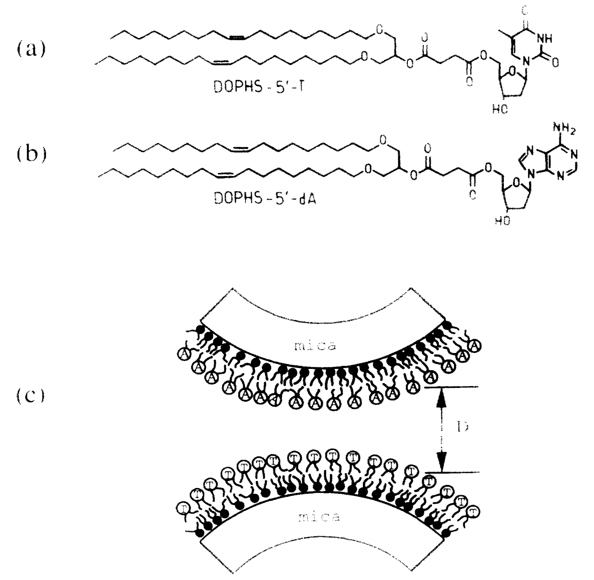

FIG. 1. Chemical structure of (a) thymidine-5′-{3-[2-(1,3-dioleoyloxypropyl) oxycarbonyl]} propionyl ester (DOPHS-5′T), (b) 2′-deoxyadenosine-5′-{3-[2-(1,3-dioleoyloxypropyl) oxycarbonyl]} propionyl ester (DOPHS-5′A). (c) Two curved mica surfaces coated with DMPE and a functionalized lipid. *D* is the distance between the two DMPE layers (abscissa in Figs. 2, 3, and 4). Here, we show the case of a layer of deoxyadenosine (A) facing a layer of thymidine (T).

DOPHS-5′A and DOPHS-5′T were synthesized by coupling the unprotected nucleosides with 2-(1,3-dioleyloxy)propyl hemisuccinate using a modified DCC/DMAP method [Lebeau and Mioskowski (unpublished data) [8]]. The SFA gives the force F between two crossed cylindrical surfaces (see Fig. 1), with a radius of curvature R as a function of the separation distance. F/R, plotted in the Figs. 2, 3, and 4, is proportional to the energy per unit area between two flat parallel surfaces. In order to ensure a perfect matching, the hydrogen bonds of the bases must

0031-9007/94/73(20)/2780(4)$06.00

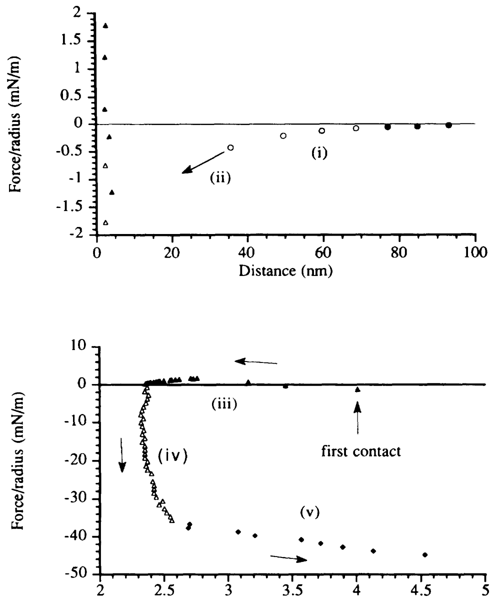

FIG. 2. Five force regimes (in this case DOPHS-5′A on both surfaces): (i) Long-range attraction (open circles), (ii) the surfaces jumped into contact (distance d_0) and flattened (arrow), (iii) the distance between the surfaces decreased slowly (filled triangles), (iv) on pulling the surfaces apart, the distance remained constant (open triangles), and (v) immediately before the surfaces separated, the distance increased progressively to d_0 (filled squares).

be oriented toward the opposing surface, while allowing enough orientational freedom. Here, the perpendicular orientation is achieved by the close packing in the layers. The flexibility is provided by a spacer between the lipid chains and the bases and by the fluidity of the monolayers due to the unsaturated chains. A Langmuir trough was used to measure compression isotherms for each surfactant, to perform the Langmuir-Blodgett depositions, and to determine the deposition ratio of the molecules from the water to the solid surface, in order to know the exact deposited surfactant density.

Each mica surface was covered with two different monolayers. First a monolayer of dimyristoylphosphatidylethanolamine (DMPE) was deposited onto the mica (at 42 mN/m) to make it hydrophobic. Then, the surfactant to be studied was deposited so that its polar headgroup faced the solution. The depositions were conducted at a pressure of 38.5 mN/m (molecular areas on water of 0.75 ± 0.02 and 0.69 ± 0.03 nm^2 for DOPHS-5′A and DOPHS-5′T, respectively). The measured deposition ratio was 1.00 ± 0.03. The surface force technique allowed us to perform measurements in pure water with different configurations: DOPHS-5′A on one surface and DOPHS-5′T on the other, hereinafter called the complementary system, and experiments with either DOPHS-5′A or DOPHS-5′T on both surfaces referred to as the symmetrical systems (see Fig. 1).

The measured force-distance profiles had the same general features in both symmetrical and complementary systems (Fig. 2). Five force regimes were observed (depicted in Fig. 2): (i) When the surfaces were brought together, no force was observed until a separation of about 60 nm was reached, below which an attraction took place. This attraction made the surfaces jump into contact as soon as the gradient of the force was higher than the force measuring spring constant, which usually occurred at a separation of 13 nm. This distance, which includes the thickness of the functionalized lipid layers (4.5 nm), corresponds to a distance of 8.5 nm between the nucleosides. (ii) The surfaces flattened immediately upon contact at a distance of $d_0 = 4.5 \pm 0.3$ nm. (iii) While the layers remained in contact, the distance decreased slowly down to 2 nm. (iv) To reverse the movement, an increasing force was applied to separate the surfaces, but the distance remained constant (to within 0.2 nm). (v) Upon further increasing the force, the separation slowly increased to d_0. Immediately after reaching d_0, the surfaces suddenly separated to a very large distance, providing a value for the adhesion free

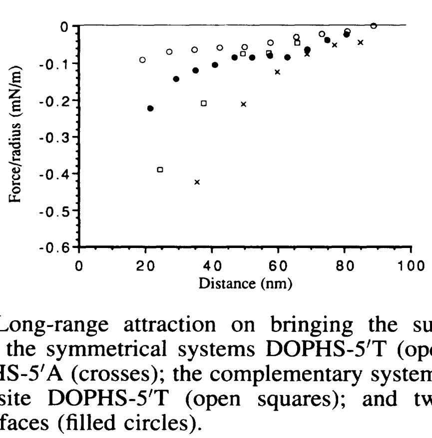

FIG. 3. Long-range attraction on bringing the surfaces together for the symmetrical systems DOPHS-5′T (open circles) and DOPHS-5′A (crosses); the complementary system DOPHS-5′A opposite DOPHS-5′T (open squares); and two DMPE coated surfaces (filled circles).

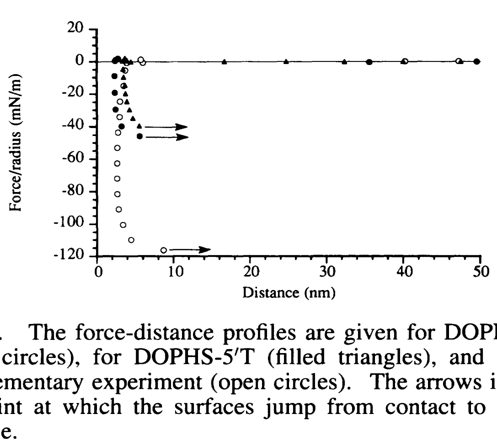

FIG. 4. The force-distance profiles are given for DOPHS-5′A (filled circles), for DOPHS-5′T (filled triangles), and for the complementary experiment (open circles). The arrows indicate the point at which the surfaces jump from contact to a large distance.

energy. The entire measurement cycle (i)–(v) could then be repeated. The repeatability of the symmetrical and complementary experiments were significantly different. For the symmetrical systems, the measurements could be repeatably performed with high reproducibility. For the complementary system, upon bringing the surfaces together following a contact and separation, a long-range erratic repulsion usually appeared. In most experiments this occurred after the initial contact and in others after a second contact. This indicates that the lipid layers were torn off the mica.

There are two significant quantitative differences between the complementary and symmetrical cases: (i) the intensity of the attraction that occurred on bringing the surfaces together, and (ii) the adhesion measured on separating of the surfaces. The range of the attraction (i.e., the distance from which it was measurable) was almost the same for the three cases (see Fig. 3), between 40 and 60 nm. However, the intensity of the force, averaged over a large number of measurements, showed significant differences. At 30 nm separation, the forces were 0.065 ± 0.03 and 0.6 ± 0.03 mN/m, respectively, for the DOPHS-5′T and DOPHS-5′A symmetrical systems and 0.21 ± 0.03 mN/m for the complementary system. The adhesion measured on separating the complementary surfaces was significantly larger that for the symmetrical cases (see Fig. 4). The force at point of separation for the symmetrical systems was −43 mN/m for DOPHS-5′T and −50 mN/m for DOPHS-5′A, while for the complementary case it was −110 mN/m.

What is the origin of the long-range attraction? It cannot be attributed to an electrostatic effect because the surfaces are not charged as proven by the symmetrical experiments in which no electrostatic double-layer repulsion was observed. Long-range attractions, flattened surfaces in contact, and strong adhesions have so far been reported for two mica surfaces made hydrophobic with a monolayer [9,10]. Depleted lipid bilayers also attract each other by hydrophobic forces from 8 nm with 15% depletion [11] and 25 nm with extended depletion [12]. To avoid this depletion effect coming from desorption, most authors saturate the solution with the same lipids. This is impossible in our case because the lipids from solution would bind to the very groups that we want to study; therefore, we have to check that the long-range attraction is not due to depletion: Since it has been shown that the larger the depletion, the stronger the attraction [12], we have measured (Fig. 3) the forces with fully depleted layers (mica coated with only a monolayer of DMPE) immersed in water. The attraction between two hydrophobic DMPE layers is much smaller than that between two layers of DOPHS-5′A (repeated measurements in numerous experiments). This shows that depletion induced forces are not strong enough to account for the long-range attraction observed between two layers of DOPHS-5′A, and therefore, the long-range attraction occurs between hydrophilic surfaces. This issue was twice cross-checked.

(i) The depositions were conducted with the maximum packing of the headgroups (collapse pressure of both functionalized lipids: 39.2 mN/m). We have made sure that this packing is conserved during our experiments by checking the insolubility of the lipids through desorption measurements. These measurements consisted in comparing the desorption of these lipids from a water-air interface with that of dioleoylphosphatidylcholine (DOPC), a lipid for which no long-range attraction was found (range of the attraction 5 nm), and which has the same aliphatic chains as DOPHS-5′A and DOPHS-5′T. After 8 h, the desorption was (11.3 ± 0.3)% for DOPC and (4.5 ± 0.3)% for DOPHS-5′A. As the forces were measured 1 h after deposition, the desorption of the lipids was insignificant. The high reproducibility in time of the force-distance profiles (symmetrical cases) confirms that no significant desorption occurred.

(ii) The nucleoside volumes can be estimated from the measured molecular areas and the value of d_0 (about 4.5 nm). Knowing that the volume of the chains [11] is 1.023 nm^3, the nucleosides with the spacer would have volumes of 0.66 and 0.53 nm^3 for DOPHS-5′A and DOPHS-5′T, respectively, values which are close to the volumes obtained by crystallography [13,14], again confirming the close packing of the layers.

The origin of the long-range attractions between hydrophobized mica surfaces has not yet been established. There is, however, an increasing evidence that it may result from a dynamic interaction between polarized domains of lipid layers on each surface [10]. The weak point of this interpretation is that one would also expect to see such polarization effects occur with hydrophilic lipid surfaces; unfortunately, up until now, no long-range attraction has been observed between hydrophilic surfaces. The present report of long-range attraction between actually hydrophilic surfaces lends some support to this description—with this interpretation the layers of DOPHS-5′A and DOPHS-5′T would have domains of fluctuating orientation which would correlate from one layer to another. Such cooperative effects could only occur in extended two-dimensional layers, and therefore, they would be unlikely to exist between complementary bases in the linear arrangement found in DNA. Further investigations will be needed to determine if this interaction is of direct relevance to DNA.

As the long-range attraction is strongest in the symmetrical system with DOPHS-5′A, there is no specific interaction at distances above 8.5 nm. However, the measured adhesion, largest for complementary system, is directly related to the preferential interaction between complementary bases. The adhesions measured between nucleosides are about 20 to 50 times larger than those observed with bilayers of usual biological lipids such as DOPC (3 mN/m). The adhesion free energy W can be calculated from the force F necessary to separate the surfaces [15]. In our case, contacting surfaces have a nontangential contact profile (that is, there is a discontinuity in the tangent at the edge of the contact zone) characteristic of strong attractions between deformable surfaces. Under these conditions (known as the JKR regime [15]), the relation between W and F is $W = 2F/3\pi R$, where R is the radius of curvature of the surfaces. The adhesion energy is then calculated to be 9.1 $\mathrm{mJ\,m^{-2}}$ for the complementary case. In principle the value 23.4 $\mathrm{mJ\,m^{-2}}$ is a lower bound, but the fact the lipid layers were not always damaged after separation shows that the actual adhesion is probably only slightly larger than this value. We can then deduce the energy of bond formation from these values and the molecular areas, assuming one-to-one molecular binding: 0.9 kcal/mole for the DOPHS-5′T/DOPHS-5′T bonds; 1.2 kcal/mole for the DOPHS-5′A/DOPHS-5′A bonds; and 2.5 kcal/mole for the DOPHS-5′A/DOPHS-5′T bonds. The mutual differences between these values for the energy of bond formation in water are in good agreement with those obtained *in vacuo* from quantum mechanical calculations [5,6]: 5.2 kcal/mole for thymine/thymine, 5.6 kcal/mole for adenine/adenine, and 7.0 kcal/mole for adenine/thymine. Bond formation energies have been measured in aqueous solvents [16,17] from thermodynamic measurements using melting temperatures in oligonucleotides. The adenine-uracyl formation energy is 2.2 kcal/mole (in RNA, uracyl plays the role of thymine), again in good agreement with the value reported here. Because, in our experiments, the anchoring points of the nucleosides are not at a fixed distance, as they are in DNA, the force measurements performed here do not involve the size effect that contributes to the preferential interaction. Hence, we conclude that the hydrogen bonds alone produce the preferential interaction without the geometrical constraints present in DNA.

The variations in the separation at contact observed for the functionalized lipids [regimes (iii) and (v) in Fig. 2] do not occur for DOPC which has the same fluid chains [7]. This unusual behavior can be understood in terms of the fluidity of the layers and the strong attraction between the nucleoside molecules. Upon bringing the layers into contact, the functionalized lipids form complexes that behave more like paraffin [18] than structured amphiphiles, and they flow like a liquid under pressure. Upon applying a force of separation, the lipids return to the contact zone.

The use of functionalized lipids in surface force experiments has allowed to extract the contribution of hydrogen bonds to base pair interactions and bond energies. The measurements show that, without the size effect, hydrogen bonds alone can produce the preferential interaction. By allowing a good control of surface density and accessibility of the sites, this method is promising for the direct measurement of weak bond energies. This is also the first report of long-range attractions between hydrophilic uncharged surfaces in water. These lipids produce unusual properties of monolayers owing to their fluid and sticky character and open an unexplored research field.

The authors are grateful to J. Meunier, J. Ninio, C. Rogier, and W. Urbach for their contributions.

[1] C. Helm, W. Knoll, and J.N. Israelachvili, Proc. Natl. Acad. Sci. U.S.A. **88**, 8169–8176 (1991).

[2] D.E. Leckband, J.N. Israelachvili, F.J. Schmitt, and W. Knoll, Science **255**, 1419–1421 (1992).

[3] B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J.D. Watson, *Molecular Biology of the Cell* (Garland Publishing, Inc., New York and London, 1989), 2nd Ed.

[4] W. Saenger, *Principles of Nucleic Acid Structure* (Springer-Verlag, New York, 1984).

[5] B. Pullman and A. Pullman, Prog. Nucleic Acid Res. Molec. Bio. **9**, 327–402 (1969).

[6] J. Wolfe, E. Perez, M. Bonanno, and J.P. Chapel, Eur. Biophys. J. **19**, 275–281 (1991).

[7] J.N. Israelachvili and G.E. Adams, J. Chem. Soc. Faraday I **74**, 975–1001 (1978).

[8] L. Lebau and C. Mioskowski (unpublished).

[9] P.M. Claesson and H.K. Christenson, J. Phys. Chem. **92**, 1650–1655 (1988).

[10] Y.H. Tsao, D.F. Evans, and H. Wennerstrom, Science **262**, 547–550 (1993).

[11] J. Marra and J.N. Israelachvili, Biochemistry **24**, 4608–4618 (1985).

[12] C.A. Helm, J.N. Israelachvili, and P.M. McGuiggan, Biochemistry **31**, 1794–1805 (1992).

[13] M.M. Radwan and H.R. Wilson, Acta Cryst. B **36**, 2185–2187 (1980).

[14] A. Grant and J. Cadet, Acta Cryst. B **34**, 1524–1528 (1978).

[15] JKR theory [K.L. Johnson, K. Kendall, and A.D. Roberts, Proc. R. Soc. London A **324**, 301 (1971)] describes geometrically the adhesion of two solids and relates the adhesion energy to the force needed to separate the surfaces. D. Maugis [J. Colloid Interface Sci. **150**, 243–269 (1992)] shows that this theory applies to the case of deformable surfaces with large adhesion.

[16] I. Jr. Tinoco, P.N. Borer, B. Dengler, M.D. Levine, O.C. Uhlenbeck, D.M. Crothers, and J. Gralla, Nature (London) **246**, 40–41 (1973).

[17] D.E. Metzler, *Biochemistry* (Academic Press, New York, 1977).

[18] M.J. Brienne, J. Gabard, J.M. Lehn, and I. Stibor, J. Chem. Soc. Chem. Commun. **24**, 1868–1870 (1989).