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Single-molecule studies of DNA and DNA–protein interactions

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Abstract

Two experiments are described that examine the properties of a single DNA molecule. The experimental system is based on optical tweezers and on a protocol that allows to attach one end of the DNA molecule to the bottom of the sample and the other to a bead of micron size. First, the reaction of the RecA protein with DNA is monitored in a particular DNA molecule. This is made possible by the fact that the DNA is locally extended by a factor of 1.5 whenever a RecA molecule binds. Second, we monitor the dynamics of a DNA molecule as it relaxes from a fully stretched state to the coil configuration.

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1. Introduction

Molecular Biology relies on biochemical methods whereby macroscopic amounts of the various compounds are being mixed yielding chemical reactions. Recently, however, a variety of techniques were developed that enable manipulation and observation of single molecules, and, in particular, biological molecules like DNA. These techniques combine high-precision optical methods with the latest developments in molecular biology. In the group of Bensimon [1], magnetic beads were used to modify the twist of dsDNA and study the resulting changes in its elastic properties. In the context of molecular motors, an optical tweezer system of nanometer precision was used to monitor the motion of kinesin on microtubules showing that it consists of steps that are 8 nm in size [2,3]. Other molecular motor systems were also studied [4,5]. The elastic properties of DNA were studied in various force ranges and salt concentrations [6–9]. In an interesting experiment, the dsDNA was slowly opened up by pulling the two strands apart. The force required to separate the strands was monitored and found to be larger in the (G–C)-rich regions of the DNA [10].

Single DNA stretching experiments were performed using micropipettes, electric or magnetic fields and optical tweezers. In our approach [9,11], we attach individual molecules that are tens of microns long (but only 2 nm thick) to the cover slip at one end and to micron size beads at the other end. Using an optical tweezer to trap the bead, this arrangement

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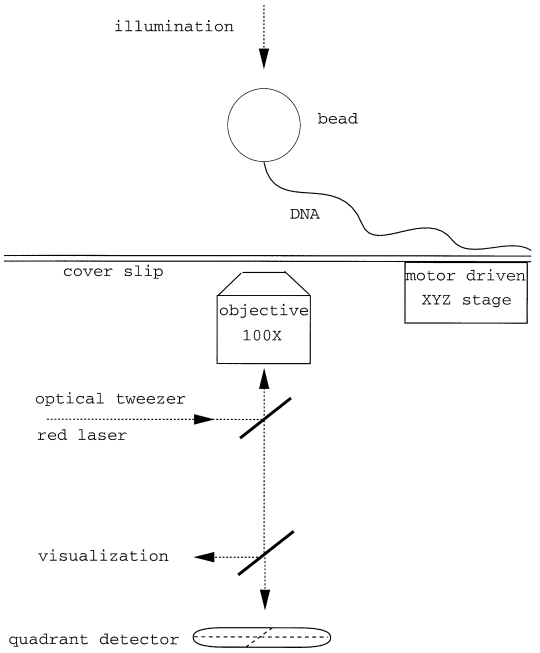

Fig. 1. The experimental setup.

allows to stretch the DNA by pulling on the bead (Fig. 1).

The optical tweezer consists of a strongly focused laser beam [12,13]. The focusing is typically achieved using a high-numerical aperture microscope objective. At the focus, the forces of the light due to diffraction on a small transparent object are all oriented inwards such that the object experiences light trapping. The trapped object can be moved with high precision by corresponding changes in the position of the focus making the optical tweezer a delicate manipulation technique.

Consequently, the tension in the DNA filament can be measured as a function of the extension, z, by monitoring the position of the bead within the optical trap. Specifically, knowing the shape of the trapping potential one can relate between the force that the DNA exerts on the bead, F_{DNA}, and the corresponding displacement of the bead from the bottom of this potential, dx. The value of dx can be obtained from the changes in the intensity of scattered light from the bead of a laser beam, an approach analogous to that used in atomic force microscopy (AFM). Such experiments yield the force–extension curve, $F_{\mathrm{DNA}}(z)$, which was found to be in agreement with the theoretical predictions from a worm like chain model. The force–extension curve and other related properties of the DNA were measured in quite a few different experimental configurations and for a wide variety of parameter values. The single DNA molecule method is presently a well-established technique that can be used as a powerful instrument to probe various processes in which the DNA plays a role.

2. DNA–protein interactions

The single DNA molecule techniques were used in order to monitor the reaction between a DNA molecule and the RecA protein (molecular weight 37.8 kDa). RecA plays an essential role in bacterial recombination [15–17]. In particular, it mediates the strand exchange reaction whereby a single-stranded DNA (ssDNA) replaces the homologous strand on a double-stranded DNA (dsDNA) [18–20]. From in vitro measurements it was found that RecA polymerizes both on single-stranded and double-stranded DNA in the presence of ATP (or ATP-γS). RecA monomers also polymerize in the absence of DNA. Depolymerization of RecA is thought to occur via hydrolysis of ATP [21–24]. Accordingly, an important control in this system is the use of ATP-γS, a non-hydrolizable form of ATP. When used it prevents RecA from disassembling. DNA is structurally modified by its association with RecA. It is stretched by a factor of 1.5 with respect to the naked form and has a twist of 20° per base pair (bp) instead of 35° in dsDNA [18,25].

In this work we directly measured the kinetics of polymerization of RecA on a dsDNA and the resulting changes in the entropic elasticity of DNA [11]. Single-molecule measurements of the role of ATP hydrolysis in RecA polymerization were performed. First, force–extension measurements were performed during the various stages of the RecA assembly process along DNA [14,11]. This allows to deduce the changes in the persistence length, A, of the DNA molecule. We find that A increases monotonically as a function of coverage such that at maximal RecA coverage it is about 4 times larger than in the naked form. Second, the length of the DNA–RecA complex is measured at small time intervals both for the case of

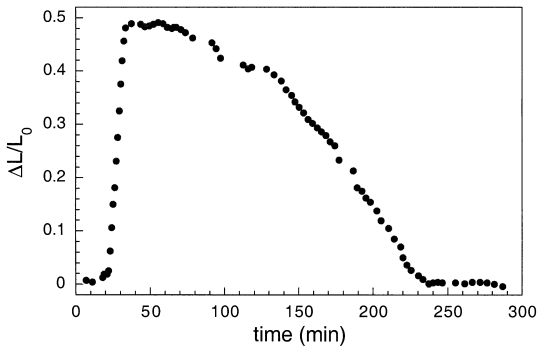

Fig. 2. The relative change in the length of DNA during the assembly of RecA on DNA in the presence of ATP (bullets).

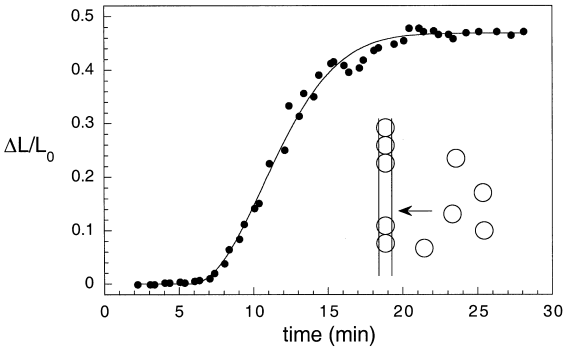

Fig. 3. The relative change in the length of DNA during the assembly of RecA on DNA in the presence of ATP-γS (bullets). The curve represents the theoretical model. A schematic illustration of the corresponding mechanism is shown in the inset.

RecA–ATP and RecA–ATP–γS reactions. One thus monitors the detailed kinetics of the reaction. For RecA–ATP, nucleation is relatively slow and the growth is fast (see Fig. 2); the opposite is observed for RecA–ATP-γS, namely, nucleation faster than growth (see Fig. 3). In parallel, this reaction was also followed at the macroscopic level via a ^{32}P isotope ATPase assay from which the [ATP]/[ADP] ratio is monitored throughout the experiment. In the ATP reaction, the length of the polymer increases first to 1.5 its original contour length and, after some time, reverses slowly to its original size by RecA depolymerization. This reversal starts for an [ATP]/[ADP] ratio of about 5 and ends at a value of about 0.25, clearly associated with ATP hydrolysis. On the other hand, the dynamics of the ATP-γS reaction lacks the depolymerization phase. Furthermore, we have shown that the dynamics of both the ATP and ATP-γS can be well described by a nucleation and growth model.

3. Can we see single-protein events ?

In the experiments we performed, the variation in the length of the DNA was obtained from the image processing of the video recording. The resolution of this approach is $\approx 0.13\,\mu$m which, in turn, corresponds to the effect of about 250 RecA monomers. Accordingly, the theoretical model of Fig. 3 relies on mean-field equations. In other words, fluctuations are too small to be of consequence. In order to study those, one needs to increase the resolution of the measurement. This can be done by either improving the image processing or using a second laser beam which scatters off the bead. This way one can reach a resolution of about 20 nm corresponding to 40 RecA monomers. At this scale, further accuracy is prevented by the thermal fluctuations of the bead. While time averaging can be used to further improve spatial resolution one is paying for this with time resolution which is equally important. The 20 nm thermal limit is related to the stiffness of the tweezer potential which cannot be made significantly larger. We are presently developing an alternative approach which will allow us to monitor the polymerization process with the accuracy of a single monomer binding event. It uses the fact that in addition to extending the length of the DNA the RecA also partially unwinds the DNA such that the twist per base pair that is 35° for naked DNA is decreased to only 20°. Therefore, during the entire polymerization process which takes about 1 h, the bead at the end of the DNA rotates about 2000 times. We suggest using aspherical beads in order to obtain the time sequence of the individual protein-binding events. The corresponding rotation can be detected either by analyzing the video recording or from a laser beam that scatters off the bead.

4. DNA dynamics

We measure the relaxation dynamics of the single DNA molecule by pulling the tethered bead with the

tweezer all the way to the escape point and turning off the laser. When the optical trap is no longer active the bead moves due to the force exerted by the DNA and against the friction from the surrounding fluid. The DNA tends to relax back to its coiled configuration in order to increase entropy. To model the force extension-curves for naked DNA, Marko and Siggia [26] used a worm like chain model. A good approximation to their result is

$$F_{\mathrm{DNA}}(z) = \frac{kT}{A}\left(\frac{z}{L} + \frac{1}{4(1-z/L)^2} - \frac{1}{4}\right) , \qquad (1)$$

where k is the Boltzmann constant, T the temperature, L_0 the contour length and A the persistence length. While the first, linear term in Eq. (1) dominates in the $z \ll L_0$ regime, the second term describes the sharp raise in the force that occurs where z approaches L_0. Eq. (1) fails when $z > 0.97L_0$. To understand the origin of the linear term in Eq. (1), note that the probability of a Gaussian chain to have an extension z is $P(z) = c\exp(-3z^2/2R_0^2)$, where c is the normalization constant and R_0 is the radius of gyration of the coil, $R_0 = 2AL_0$. The corresponding free energy E is purely entropic, $E = -TS = -kT\ln P(z) = 3kTz^2/4AL_0$ and $F = \mathrm{d}E/\mathrm{d}z = 3kTz/2AL_0$. The different prefactor is accounted for by the part of the second term in Eq. (1) that is linear in z and which can be obtained by expanding in a Taylor series.

On the other hand, the inertia of the beads turns out to be six orders of magnitude smaller than the other forces involved and therefore

$$F_{\mathrm{DNA}} = F_{\mathrm{Stokes}} = 6\pi\eta a\left(1 + \frac{9}{16}\frac{a}{h}\right)v_{\mathrm{b}} , \qquad (2)$$

where a is the radius of the bead, h is the height of its center over the cover slip, η is the viscosity and v_b is the velocity. The correction to the Stokes law is due to the proximity to a boundary, namely, the bottom of the sample. The relaxation dynamics of the DNA–bead system is monitored via image processing of the video recording at 10 frames/s. The time scale for the motion of the bead, $\tau_{\mathrm{b}} \approx 5\,\mathrm{s}$, is much larger than the equilibration time for the DNA (the Zimm time), $\tau_{\mathrm{Z}} \approx 0.1\,\mathrm{s}$, and, therefore, the DNA relaxation is expected to be quasistatic. That is, during relaxation DNA is passing only through equilibrium stretched states [27]. One can use Eq. (1) for F_{DNA} to show that a simple rescaling of the variables makes the recoil dynamic dependent only on A. Replacing v by $\mathrm{d}z/\mathrm{d}t$ in Eq. (2) and rescaling both the extension, $z' = z/L_0$, and the time, $t' = t/L_0$, the differential equation for $z'(t')$ becomes dependent only on the persistence length A. Moreover, the value of t' during which the DNA relaxes to some fraction of its length, z', $t'_{z'}$, is proportional to the persistence length, $t'_{z'} = c(z')A$. In Fig. 4, the data points correspond to two different DNA molecules with different L_0's and within experimental error they coincide.

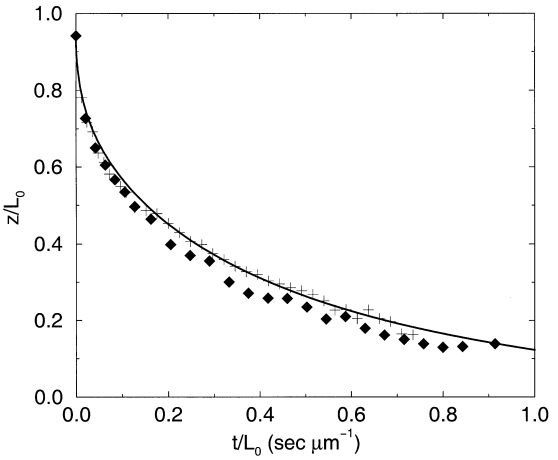

Fig. 4. Recoil dynamics of DNA. Two different experiments (diamonds and plusses) and the best fitting theoretical curve for the plusses experiment which corresponds to $A_D = 152$.

The equation of motion of Eq. (2) can be explicitly integrated to yield the theoretical $z'(t')$. The best fitting $z'(t')$ with respect to the persistence length, $A_{\mathrm{D}} = 152 \pm 2\,\mathrm{nm}$, is also shown in Fig. 4. This value is three times larger than that obtained from static force measurements [28] and biophysical methods [29], $A \approx 50\,\mathrm{nm}$. This discrepancy could be due to either (1) dynamical effects of DNA relaxation which are being neglected in Eq. (2) or (2) friction between the DNA and the bottom of the sample. In order to reduce the effect of the friction with the bottom, we also extract the persistence length only from the initial part of the recoil during which the DNA is less likely to come in contact with the bottom of the sample. Indeed, a rather sharp change in A_{D} is found for $t' \approx 0.2$. For $t' < 0.2$ the best fit corresponds to $A_{\mathrm{D}} = 128 \pm 4\,\mathrm{nm}$. Nevertheless, this value is still significantly larger than A indicating that non-stationary effects need to be closely examined in order to understand the dynamics of the DNA recoil.

5. Conclusions

The two single DNA molecule experiments that are described in this paper are examples from a relatively new field, namely, that of single molecule biophysics. The advantages of the single-molecule methods are at least threefold. First, the size of the system is typically in the few nanometers range. The ability to manipulate and modify objects of this size is important for miniaturization technologies [30]. In turn, such technologies are used for electronic devices, biological or medical instruments and hybrid systems where traditional electronics is mixed with organic components. Second, such methods are characterized by high accuracy whereby very fine effects are being observed. In particular, forces of less than a single pN are measured with high precision. This allows, for example, to monitor the unfolding of a chemical reaction between only two molecules. Finally, single-molecule methods represent a direct observation of the phenomenon in question. In other words, there is no loss of information due to averaging over 10^{23} molecules as is done in biochemistry.

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References

[1] T.R. Strick, J.-F. Allemand, D. Bensimon, A. Bensimon, V. Croquette, Science 271 (1996) 1835.
[2] M.J. Schnitzer, S.M. Block, Nature 388 (1997) 386.
[3] K. Svoboda, C.F. Schmidt, B.J. Schnapp, S.M. Block, Nature 365 (1993) 721.
[4] J.T. Finer, R.M. Simmons, J.A. Spudich, Nature 368 (1994) 113.
[5] D.A. Schafer, J. Gelles, M.P. Sheetz, R. Landick, Nature 353 (1991) 444.
[6] S.B. Smith, L. Finzi, C. Bustamante, Science 258 (1992) 1122.
[7] R.M. Simmons, J.T. Finer, S. Chu, J. Spudich, Biophys. J. 70 (1996) 1813.
[8] M.D. Wang, H. Yin, R. Landick, J. Gelles, S.M. Block, Biophys. J. 72 (1997) 1335.
[9] G.V. Shivashankar, G. Stolovitzky, A. Libchaber, Appl. Phys. Lett. 73 (1998) 291.
[10] B. Essevaz-Roulet, U. Bockelmann, F. Heslot, Proc. Natl. Acad. Sci. USA 94 (1997) 11 935.
[11] G.V. Shivashankar, M. Feingold, O. Krichevsky, A. Libchaber, Proc. Natl. Acad. Sci. USA 96 (1999) 7916.
[12] A. Ashkin, J.M. Dziedzic, J.E. Bjorkholm, S. Chu, Opt. Lett. 11 (1986) 288.
[13] A. Ashkin, Biophys. J. 61 (1992) 569.
[14] J.F. Leger, J. Bourdieu, D. Chatenay, J.F. Marko, Proc. Natl. Acad. Sci. USA 95 (1998) 12 295.
[15] A.I. Roca, M.M. Cox, Crit. Rev. Biochem. Mol. Biol. 25 (1990) 415.
[16] S.C. Kowalczykowski, A.K. Eggleston, Ann. Rev. Biochem. 63 (1994) 991.
[17] M.M. Cox, Trends Biochem. Sci. 19 (1994) 217.
[18] P. Howard-Flanders, S.C. West, A. Stasiak, Nature 309 (1984) 215.
[19] E. Cassuto, P. Howard-Flanders, Nucleic Acids Res. 14 (1986) 1149.
[20] S.M. Honigberg, C.M. Radding, Cell 54 (1988) 525.
[21] S.L. Brenner, A. Zlotnick, J.D. Griffith, J. Mol. Biol. 204 (1988) 959.
[22] S.L. Brenner, A. Zlotnick, J.D. Griffith, J. Mol. Biol. 216 (1990) 949.
[23] B.F. Pugh, M.M. Cox, J. Mol. Biol. 203 (1988) 479.
[24] J.F. Lindsley, M.M. Cox, J. Mol. Biol. 205 (1989) 695.
[25] M. Takahashi, B. Norden, Adv. Biophys. 30 (1994) 1.
[26] J. Marko, E. Siggia, Macromolecules 28 (1995) 8759.
[27] T.T. Perkins, S.R. Quake, D.E. Smith, S. Chu, Science 264 (1994) 822.
[28] C.G. Baumann, S.B. Smith, V.A. Bloomfield, C. Bustamante, Proc. Natl. Acad. Sci. USA 94 (1997) 6185.
[29] P.J. Hagerman, Annu. Rev. Biophys. Biophys. Chem. 17 (1988) 265.
[30] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, Nature 391 (1998) 775.