

**Proprietes elastiques d'une molécule d'ADN simple brin,
et interactions ADN hélicases à l'échelle de la molécule
unique**

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RecQ Helicase: Single Molecule Activity and Step Size

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DNA helicases are enzymes capable of unwinding double-stranded DNA (ds-DNA) to provide the single-stranded DNA (ss-DNA) required in replication, recombination and repair [1–3]. These molecular motors use the hydrolysis of ATP to unwind and translocate along DNA. Among helicases, members of the RecQ family (spanning a wide variety of organisms: bacteria, yeast, fly and humans) play an essential role in the maintenance of genomic integrity. In humans, mutations lead to Bloom and Werner's syndromes (increased cancer susceptibility and premature aging respectively) [4]. A mechanistic understanding of these enzymes has proved difficult to achieve in bulk measurements. Here we use a magnetic trap technique to monitor in real time the behaviour of RecQ. Our observations show that RecQ works in bursts of unwinding followed upon its dissociation by fast rehybridization. We have measured RecQ's rate of unwinding, processivity and on-time as a function of ATP concentration and estimated its step-size. We find its rate, processivity and efficiency to be much higher than previously estimated in bulk [5]. These experiments shed new light on the activity of helicases.

We have characterized the behaviour of RecQ DNA-helicase in an experimental configuration similar to the one used to study DNA polymerases [6, 7]. Briefly, a single DNA molecule is anchored at one end to a magnetic bead and at the other end to a glass surface. By approaching permanent magnets placed above the sample, we control the force F , applied to the molecule. Using video microscopy, we monitor in real time the molecule's extension l [8]. As, at $F > 5$ pN, ss-DNA stretches more than the corresponding ds-DNA [6], we can study helicase driven unwinding by measuring the lengthening of the molecule (~ 0.13 nm/bp at $F = 35$ pN)(see Fig.1).

Transiently stretching to 65 pN a nicked DNA molecule partially melts it [9] and provides the ds/ss DNA junction required for initiation of helicase activity, presumably in a 3'→5' direction [10].

At $30 < F < 50$ pN and only in the presence of both RecQ and ATP ($[\text{RecQ}] \sim 0.2 \text{ nM} < k_d^{-1} \sim 10 \text{ nM}$) one observe bursts of increasing DNA extension resulting from the helicase unwinding activity (*i.e.* the transformation of ds-DNA to ss-DNA) followed by abrupt decrease in DNA extension due to rapid rehybridization of the molecule as RecQ dissociates from its substrate (see Fig.1). These bursts are separated by periods of inactivity, typical of single molecule behaviour [11]. Since ds-DNA is energetically more stable than its separated strands it is rapidly regenerated from the nucleation point provided by the fork following RecQ dissociation. On the other hand in the presence of a RecQ complex rehybridization is suppressed at $F > 30$ pN, due to a kinetic barrier to nucleation resulting from the mismatch between the stretched and unstretched bases. Thus in these experiments, the force plays a role akin to SSB proteins in stabilizing the displaced ssDNA against rehybridization. We therefore characterized the behaviour of RecQ in presence of saturating amounts of SSBs proteins,

($100 \text{ nM} \gg k_d^{-1} \sim 6 \text{ nM}$), in order to be able to detect the activity of the helicase at much lower force. Indeed, the helicase activity could be observed under those conditions, at forces ranging from ~ 35 to ~ 12 pN, though rehybridization upon dissociation of the helicase complex still occurred (see Fig.1). Experiments at forces less than 10 pN are much less precise due to reduced signal (the change in extension between ss and ds-DNA is smaller) and increased noise (increased fluctuations from a bead under less tension).

From these data one can extract a number of important dynamical variables. The local rate of RecQ unwinding, v , is given by the positive slope of a burst of activity. The helicase processivity P is given by the height of a burst, and the on-time τ_{on} by its duration (see Fig.1). The distributions of v , P and τ_{on} are shown in Fig.2, at $F = 35 \text{ pN}$ and $[\text{ATP}] = 500 \mu\text{M}$. While the helicase rate of unwinding is approximately constant (25% variation about its mean), its processivity and on-time follow, as expected, a Poisson distribution.

We have measured the mean rate $\langle v \rangle$, processivity $\langle P \rangle$ and on-time $\langle \tau_{on} \rangle$ as a function of $[\text{ATP}]$, at $F \sim 35 \text{ pN}$. A first-order Michaelis-Menten (MM) kinetics, fits very well the data for $\langle v \rangle$ ($K_M = 40 \pm 5 \mu\text{M}$ and $V_{max} = 106 \pm 10$ base-pairs (bp)/sec) and $\langle P \rangle$ ($K_M = 50 \pm 6 \mu\text{M}$ and $P_{max} = 780 \pm 50$ bp) (see Fig.3). On the other hand $\langle \tau_{on} \rangle = 7 \pm 0.8$ s does not vary significantly with $[\text{ATP}]$, and thus $\langle P \rangle = \langle v \rangle \langle \tau_{on} \rangle$ (see Fig.3). To check that our results were not sensitive to the applied force, we have conducted experiments at saturating RecQ concentration to prevent rehybridization of the displaced strand, taking advantage of the SSB like behaviour of the helicase, in order to measure the velocity as a function of the force applied. (In the case of the experiments conducted with high SSBs, it is hard to know whether RecQ or SSB binds on the formed single strand, and therefore it is hard to

estimate the elasticity the molecule should have). The experiments revealed that the velocity of the helicase changed by less than 20% as the force was reduced from 35 to 12 pN.

We also estimated the step size δz of the helicase, *i.e.* the number of bases opened per enzymatic cycle using an analysis of the extension noise δl^2 in a burst of unwinding [12]. This noise is caused both by the random nature of the stepping process and by the bead's fluctuations. At low frequency the stepping noise spectrum increases as f^{-2} and overcomes the brownian fluctuations spectrum (which is frequency independent at low f). Assuming a Poisson distribution for the time between steps, the step-size can be deduced from the amplitude of the low frequency noise $\lim_{f \rightarrow 0} \delta l^2(f) = \delta z(v)/2\pi^2 f^2$ [13]. From the measurements (see Fig.4), we estimate the value of the step size $\delta z = 1.14(\pm 0.17)$ nm corresponding to 9 (± 2) bp unwound per enzymatic cycle [14–16]. Because of the smaller velocity and processivity at low [ATP], we could estimate δz only at saturating [ATP], and sufficiently long bursts of unwinding.

A striking feature is that those results are different from bulk measurements on RecQ [5]. There, the helicase activity was reported to be cooperative in [ATP] (with a Hill coefficient of 3.3), and an unwinding rate of $V_{bulk} = 2.3$ bp/sec/monomer [5]. Further our estimated step size for RecQ, close to its maximal theoretical value [1, 20], is larger than the one deduced from bulk measurements (0.4 - 1 bp per ATP hydrolysed) [1], (or 2-4 bp unwound per successful enzymatic step [21]). It is conceivable that the high velocity (~ 100 bp/s) reported here, is due to the force applied on the molecule. However, we have verified that the velocity is largely independent of the force.

Our observations of the activity of a single RecQ helicase characterized by bursts of unwinding followed by rapid rehybridization (at velocity v_-) suggest that bulk experiments, which measure the average number of fully unwound DNA (at rate $V_{bulk} \ll v$), may not reflect the maximal activity of the enzyme. Our data suggest that the kinetics of unwinding by a single helicase are formally similar to the dynamic instability of microtubules [22, 23]. If τ_{off} is the mean time between two bursts of activity, it can be shown [24], that the resulting dynamics are characterized by two regimes: a finite unwinding regime with $V_{bulk} = 0$ at low [ATP] in average, and a regime at high [ATP] with an unwinding rate: $V_{bulk} = (\tau_{on}v - \tau_{off}v_-)/(\tau_{on} + \tau_{off})$. Indeed bulk data are well fitted by this model (data not shown) suggesting that the transition between these regimes at finite ATP has been misinterpreted as implying ATP cooperativity. On the other hand single molecule data, measuring v , are well fitted by first order MM kinetics. It is worth noting that single molecule experiments conducted on RecBCD, (a nuclease/helicase complex), are however in agreement with the bulk measurements conducted on that complex

[17–19]. This helicase complex possesses a nuclease activity that prevents any rehybridization, thus in that case $V_{bulk} = v$. It is also worth noting that, as we found for RecQ, the processivity of RecBCD varies linearly with the velocity, with an [ATP] independent $\tau_{on} \sim 50$ s. As no rehybridization is possible in the case of that complex, it is not surprising that bulk experiments, as well as single molecule experiments on that particular enzyme give the same results.

Finally, the large increase in the low frequency noise when the enzyme is active is inconsistent with a small enzymatic step-size. In fact the value deduced from our data, implies that the enzyme has an efficiency close to 100 %. It is likely that the low efficiency deduced from bulk measurements is due to the competition between unwinding and rehybridization. This has sometimes however been interpreted as enzymatic slippage [1].

These single molecule experiments give a different perspective on the behaviour of DNA helicases and their efficiency as molecular motors. It appears that a more accurate description of the *in vitro* activity of the protein should take into account the rehybridization events following unwinding.

Materials and methods

11 kbp DNA was labeled at its extremities with biotin and digoxigenin and mixed with streptavidin-coated magnetic bead (4.5 μ m, Dynal) [6]. The bead-DNA construct was then incubated on an anti-digoxigenin coated glass surface, previously incubated with t-RNA (Sigma) to reduce non-specific interactions. **Helicase experiments** were performed at 25°C in a 20 mM Tris buffer HCl containing 25 mM NaCl, 3 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT. RecQ was purified and assayed by both DNA-dependent ATP hydrolysis and helicase activity. ATP (Amersham) and SSB (USB) were added to the final concentrations indicated.

Bead tracking and force measurements were performed on an inverted microscope as previously described [8]. By tracking the 3D position of the tethered bead [25, 26] the extension $l = \langle z \rangle$ of the molecule was measured, with an error due to Brownian fluctuations of $\sim 5nm^2.Hz^{-1}$ (at $F = 30pN$). The bead's transverse fluctuations $\langle \delta x^2 \rangle$ allow a determination of the applied force via the equipartition theorem: $F = \frac{k_B T l}{\langle \delta x^2 \rangle}$. F has been measured with a 15% accuracy. To eliminate microscope drift, differential tracking with a second bead glued to the surface was performed.

Data processing. The determination of the helicase unwinding rate requires measuring the elongation vs. time: $l(t)$. The number of basepairs unwound are given by: $N(t) = [l(t) - l(0)]N_{tot}/\Delta l(F)$, where N_{tot} is the total number of base-pairs in the dsDNA strand and $\Delta l(F)$ the total change in extension between dsDNA and ssDNA at force F . The local velocity v_i of the helicase was determined as previously described [6] by fitting

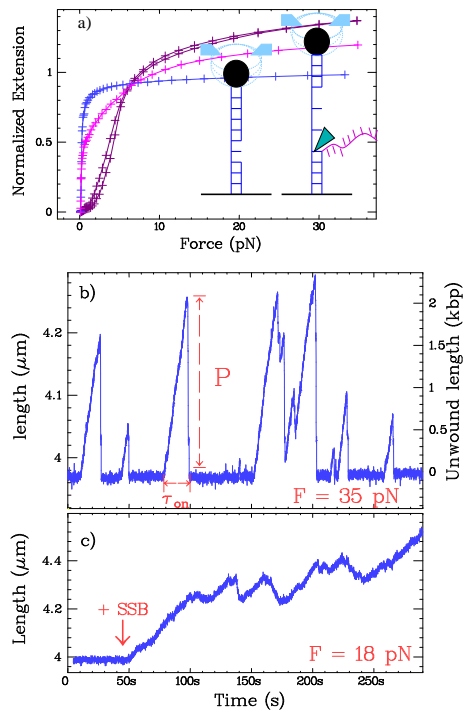


FIG. 1: Experimental set-up and unwinding signals. **a-** Extension of a stretched ds-DNA (blue), ss-DNA (red) and a partially ds-ss DNA molecule (magenta curve) (normalized to the crystallographic length of ds-DNA). Superimposed on these data is a schematic representation of the experimental set-up. Magnets (in blue) placed a few mm above the sample apply a force on the molecule. **b-** Typical unwinding signal obtained upon addition of 0.25 nM of RecQ ([ATP]= 500 μ M, $F = 35$ pN, [SSB]= 0). **c-** Typical signal obtained at [ATP]= 500 μ M, $F=18$ pN, [recQ]= 50 nM upon addition (arrow) of 100 nM SSB. Because of the complex kinetics of SSB binding to ss-DNA, which modifies DNA length, we cannot reliably convert the measured changes in extension to a number of base-pairs.

the data in an extension burst $l(t)$ to a polygon $\{l_i, t_i\}$ of $i = 1, \dots, M$ vertices and computing the local slope $v_i = (l_i - l_{i-1}) / (t_i - t_{i-1})$.

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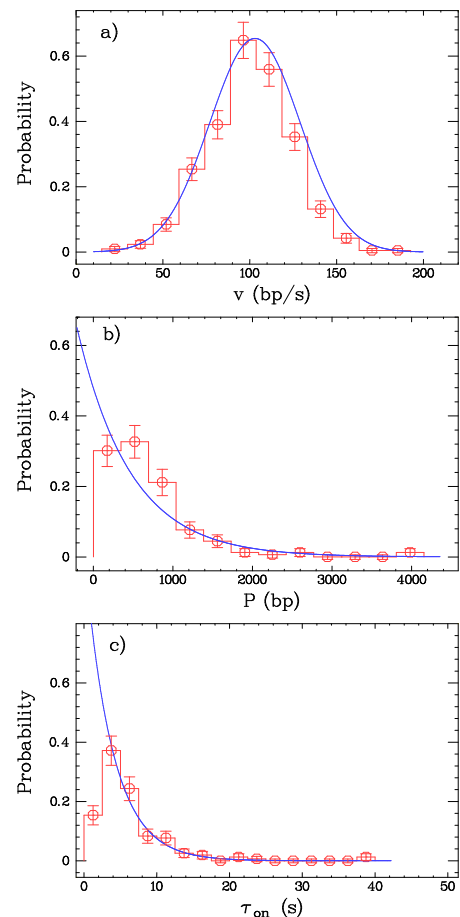


FIG. 2: Histograms of the enzymatic rate v (**a**, $n=532$), processivity P (**b**, $n=157$) and on-time τ_{on} (**c**, with $n=157$, where n is the number of points used to draw the histogram) ([ATP]=500 μ M, $F = 35$ pN). The data were fitted (χ^2 minimization) in **a** to a Gaussian distribution of mean value $\langle v \rangle = 102$ bp/s and $\sigma = 26$ bp/s, in **b** and **c** to a Poisson distribution with mean value $\langle P \rangle = 692 \pm 80$ bp and $\langle \tau_{on} \rangle = 5 \pm 0.7$ s respectively. Error bars represent the statistical error in the bins.

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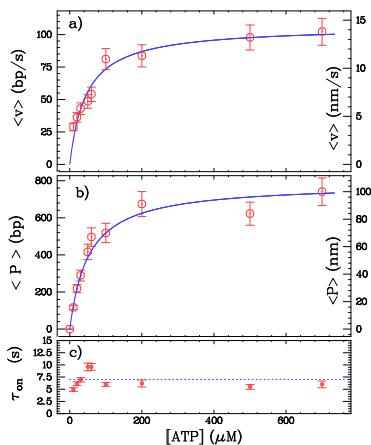


FIG. 3: ATP-dependence of the average rate, $\langle v \rangle$, processivity $\langle P \rangle$ and on-time $\langle \tau_{on} \rangle$ of RecQ ($F=35$ pN). Each point is the average over histograms obtained on several DNA molecules. **a-** $\langle v \rangle$ vs [ATP]. The data were fitted (χ^2 minimization) with a first order MM kinetics $\langle v \rangle = V_{max}[ATP]/(K_M + [ATP])$ of $V_{max} = 106 \pm 10$ bp/s and $K_M = 40 \pm 5 \mu M$ **b-** $\langle P \rangle$ vs [ATP]. The data were fitted with a first order Michaelis-Menten kinetics (χ^2 minimization) of $P_{max} = 780 \pm 8 bp$ and $K_M = 50 \pm 6 \mu M$ **c-** $\langle \tau_{on} \rangle$ vs [ATP]. The average over all [ATP] conditions is $\langle \tau_{on} \rangle = 7 \pm 0.8s$.

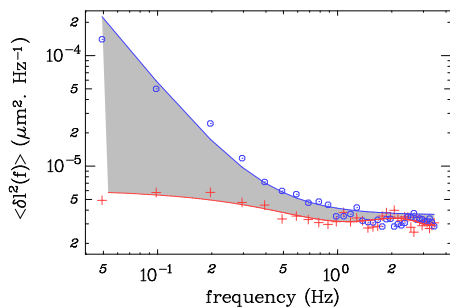


FIG. 4: Evaluation in the frequency domain of the step size of RecQ. Average extension power spectrum of 47 bursts of unwinding activity (blue) and 48 experimental noise traces recorded when the enzyme is inactive (red curve) ($[ATP] = 1mM$, $F = 30pN$, $\langle v \rangle = 80$ bp/s). The blue curve (o) exhibits a low frequency noise increasing as f^{-2} due to the random stepping motion of recQ. The data were fitted to $\delta l^2 = \delta z \langle v \rangle / 2\pi^2 f^2 + b$, leading to an estimation of the step size $\delta z = 1.14 \pm 0.17$ nm, i.e. 9 ± 2 bps. The quality factor $Q \sim 10 \gg 1$ is large enough to reliably estimate δz [13].

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