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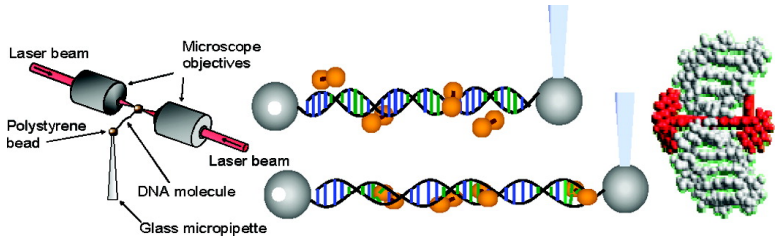

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Mechanically Manipulating the DNA Threading Intercalation Rate

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Studies of the interactions of small molecules with DNA are essential for designing and developing better probes for diagnosis and drugs for cancer therapy. Since $[Ru(phen)_3]^{2+}$ (phen = 1,10 phenanthroline) was introduced in 1984,[1] ruthenium complexes have played an important role in the design of DNA binding small molecules. One goal in the construction of molecules that target the reproduction of tumor cells is that the developed complexes should have a high affinity to DNA as well as slow dissociation from DNA.[2] Several modifications have been made to ruthenium complexes to improve their DNA-binding properties. For example, the replacement of one of the phen moieties with dppz, (dppz = dipyrido[3,2-a:2′,3′-c]phenazine) increases the binding constant by 3 orders of magnitude.[3,4]

The binding kinetics are significantly altered by covalently linking two $[Ru(phen)_2dppz]^{2+}$ molecules to form binuclear ruthenium complexes, one such example being the semirigid Δ,Δ-[μ-bidppz$(phen)_4Ru_2]^{4+}$ [bidppz = 11,11′-bi(dipyrido[3,2-a: 2′3′-c]phenazinyl)],[5] which shall here be denoted as **ΔΔ-P** (Δ denoting the ruthenium coordination to be right-handed). This dumbbell shaped binuclear ruthenium complex has been shown to have extremely slow DNA binding kinetics. Initially the complex was believed to interact with DNA exclusively by binding to the grooves, but it was later shown that the dominant binding mode was one of threaded intercalation (Figure 1), in which the bridging bidppz ligand is sandwiched between adjacent DNA base pairs.[6] To reach this final state the propeller-like bulky end of this molecule must thread through the DNA base pairs, which transiently requires the opening of base pairs, i.e., local DNA melting, to an unknown extent. In bulk experiments this process relies on rare thermal fluctuations to open up and allow threading, which results in an extremely long time to reach the final threaded state.[6−8] Once the complex is bound by threading intercalation, it exhibits very slow dissociation[7,9] and hence it is an excellent model compound for therapeutic applications.

In the current studies, we use optical tweezers[10] to stretch single lambda DNA molecules in the presence and absence of the binuclear threading intercalator **ΔΔ-P**. We have previously shown that DNA intercalation can be quantified with high precision and sensitivity by measuring the increase in DNA contour length with intercalator concentration.[11] In the case of simple intercalators, ligand association and dissociation occurs rapidly on the time scale of a DNA stretching experiment, such that equilibrium ligand−DNA association constants could be obtained as a function of force, and the equilibrium constant increased exponentially with applied force.[11]

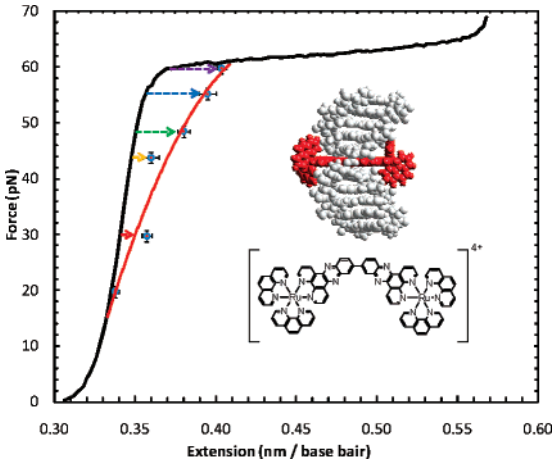

Figure 1. Force extension measurements of single DNA molecule in the presence of 2 nM binuclear complex **ΔΔ-P** (red circles) and in the absence of complex (black). The red line is a fit to guide the eye. These experiments were performed at 20 °C in 10 mM TRIS buffer (100 mM Na^+, pH 8, three or more measurements for each point). The broken arrows indicate the lengthening of the DNA shown in Figure 2. Inset: A cartoon of the predicted structure of **ΔΔ-P** (red) threaded through DNA bases and its chemical structure.

In contrast, **ΔΔ-P** exhibits very slow association and dissociation, such that the increase in DNA contour length exhibited in DNA force-extension curves do not represent the equilibrium DNA−intercalator complex. This slow binding is consistent with bulk experiments, which showed that many hours were required for DNA threading to be observed at room temperature and low ionic strength.[6] An equilibrium DNA force-extension curve in the absence of intercalator is shown in Figure 1. In order to obtain equilibrium force-extension curves for DNA in the presence of **ΔΔ-P**, we have measured the DNA extension at constant force until it reaches equilibrium,[12] which is on the order of hours at room temperature. As shown in Figure 1, the final equilibrium extension increases significantly with force at a constant intercalator concentration of 2 nM. The arrows in the figure indicate how the extension is changing with time, and the resulting extension vs time at constant force is shown in Figure 2. These constant force measurements allow us to directly measure the rate of DNA threading intercalation as a function of force. The data in Figure 2 fit well to a single-exponential dependence with time, and the resulting time constants are presented in Figure 3.

The slow rate of **ΔΔ-P** /DNA intercalation in combination with its exponential dependence on applied force suggests that the ligand threading rate is limited by the very small probability of melting of one or more DNA bp, which is required before threading can occur. The applied force favors the more extended melted state of

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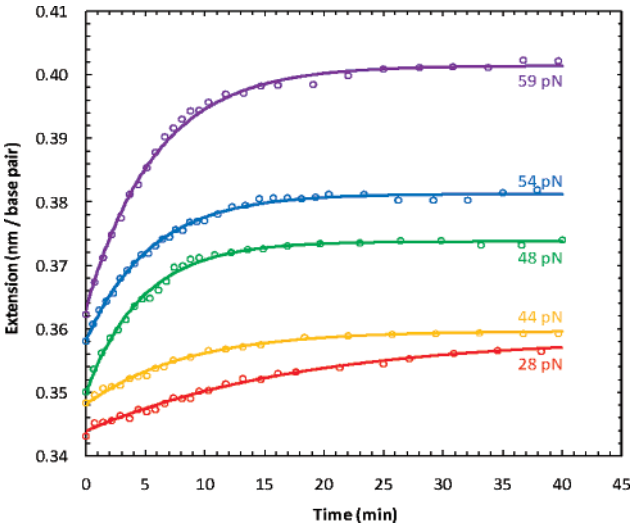

Figure 2. Representative (from three or more measurements) constant force extension data with 2 nM ΔΔ-**P** at different constant forces (open circles) and their corresponding single-exponential fits (solid lines). Experimental conditions: 20 °C, 10 mM TRIS (100 mM NaCl, pH 8).

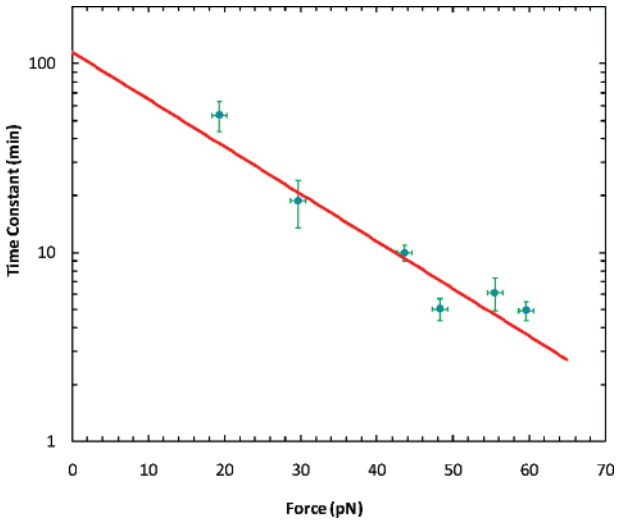

Figure 3. Time constants for threading of the binuclear complex ΔΔ-**P** into DNA. Circles are experimental results from three or more measurements with error bars determined from the standard deviation. Solid line is fit to an exponential dependence on force using a χ^2 analysis that includes the error bars for each point.

DNA, thereby decreasing the melting free energy by $Fn\Delta x$ and increasing the probability of melting by the factor $e^{Fn\Delta x/k_BT}$.[13] Here F is the applied force, and Δx is the difference in length per base pair between ssDNA and dsDNA at that force, which is constant over the force range studied,[14] and n is the number of transiently melted base pairs required for ligand threading. Therefore, the threading time constants obtained from the data in Figure 2 are expected to decrease exponentially with applied force as $\tau = \tau_0 e^{-Fn\Delta x/k_BT}$.

The result from fitting our measured force-dependent time constant to the predicted exponential behavior is shown as a solid line in Figure 3. The data fit well to this dependence, and the resulting change in DNA length upon melting required for the threading is 0.239 ± 0.025 nm. Therefore, assuming an average Δx value of 0.22 nm for DNA in the linear approximation region,[10,14] we have determined that only 1.08 ± 0.11 bp must be melted by thermal fluctuations in order for the binuclear ruthenium complex to thread between the bases and intercalate into the DNA. If we extrapolate our results to zero force, we obtain a reaction time constant of 120 ± 30 min.

Previous measurements of threading intercalation kinetics for ΔΔ-**P** intercalating into polydisperse calf thymus DNA, obtained using spectroscopic methods in the temperature range 40−60 °C, have shown a two-phase process, with the first phase at least 6 times faster than the second.[6,7] The observation of two rates in those experiments may be due to processes not detected by DNA stretching, such as ligand rearrangement after binding or conformational changes in the DNA. In the Supporting Information, we show that measurements of the enthalpy of the rate-limiting threading step from previous high-temperature spectroscopic measurements are consistent with the enthalpy of one base pair opening in the middle of the DNA duplex.

To conclude, we have demonstrated that the DNA threading rate into a single DNA molecule can be directly measured at constant force using optical tweezers. Our results show that the known dependence of DNA destabilization upon force can be used to predict the dependence of the threading rate on force. Because we manipulate the measurable physical parameters of length and force, we are able to directly determine the physical length change responsible for lowering the reaction barrier. From this data, we find that only one base pair must be melted in order for threading intercalation of ΔΔ-**P** to occur. This result is consistent with the minimum steric requirements for threading based on structural considerations.

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Supporting Information Available: Comparison of high-temperature spectroscopic data for DNA threading with DNA stretching results. This material is available free of charge via the Internet at http://pubs.acs.org.

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