Cisplatin Changes the Mechanics of Single DNA Molecules**

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Recent experiments have revealed that on a molecular scale not only the energies, but also the forces involved in biological and chemical processes are important structural and functional parameters;[1] for example, enzymes involved in gene regulation and gene expression exert piconewton (pN) forces on DNA to fulfill their biological function.[2] Stretching experiments with individual molecules have shown that double-stranded DNA (dsDNA) can be overstretched to almost twice its contour length without rupturing.[3–5] The force versus distance curves (see Figure 1 A) show a distinct plateau around 70 pN where the molecule is forced into a structural transition from its native B conformation to an overstretched state, called S-DNA. This transition is highly cooperative in native DNA. If the molecule is stretched beyond the B-S transition a second structural transition can be observed during which the double helix is split into two single strands. The flattening of the force curves in this melting transition also indicates that a certain degree of cooperativity is involved in the process. The molecule may recombine to its double helical conformation upon relaxation.[6, 7] The extent of hysteresis (see Figure 1 A) between stretching and relaxation depends on the experimental parameters such as the pulling velocity.

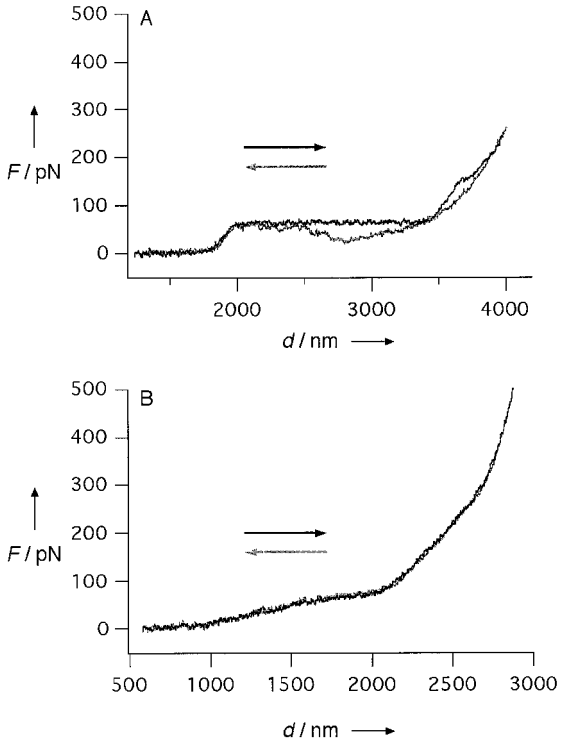
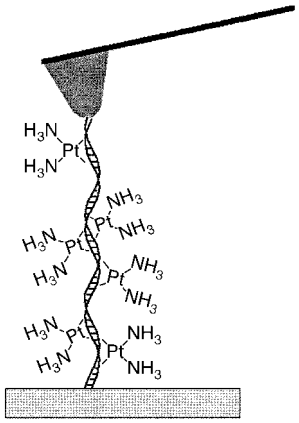


Figure 1. The force versus distance curves obtained on untreated λ-digest DNA (A) and after reaction with cisplatin (B). Here and in the following figures the black curve shows the extension and the gray gurve the relaxation traces.

Cisplatin is one of the most common anticancer drugs, and its interactions with DNA have been studied intensively for many years.[8] Cisplatin is known to form cross-links in DNA, where it preferentially binds to the N7 atoms of guanine bases. The most common bifunctional products with dsDNA are the intrastrand cross-links between two guanine bases, which can either be neighboring or separated by any other base (G*G* or G*XG*, respectively), and the intrastrand cross-links between a guanine base and an adenine base (5′-A*G*-3′). Cisplatin can also form interstrand cross-links between two guanine bases at a GC sequence.

Here we report the direct observation of structural changes induced by cisplatin as measured by single-molecule force spectroscopy.[9] Individual molecules with different base composition attached between an atomic force microscopy (AFM) tip and a gold substrate were stretched (Scheme 1) and the force versus distance curves were recorded. The experiments revealed significant changes in the mechanical properties of the DNA molecules after platination as compared to untreated molecules. These changes strongly depend on the sequence of the stretched molecules and we attribute them to the formation of bifunctional products between cisplatin and DNA, namely interstrand and intrastrand cross-links.

The force versus distance curve taken on a single molecule of λ-digest DNA after reaction with an excess of cisplatin is shown in Figure 1 B. Here all of the adducts described above may have formed. The B-S transition is significantly less cooperative than in native DNA: instead of a flat plateau, a steady rise in force up to (73 ± 5) pN is observed. At forces of (318 ± 22) pN a second kink appears, but there is almost no flattening of the curve as is observed in the melting transition of untreated molecules.[6, 7] The forces at which these features occur are independent of the pulling velocity within the experimentally accessible range (200 nm s^{-1} to 4 μm s^{-1}). Moreover, the relaxation traces are virtually indistinguishable from the extension traces, even if the molecules are stretched up to forces of more than 500 pN. This observation shows that cisplatin inhibits a permanent mechanical separation of the double helix.



Scheme 1. An individual platinated DNA molecule is stretched between an AFM tip and the substrate.

The same kind of stretching experiments were also carried out on synthetic dsDNA molecules of specific

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[**] Helpful discussions and support from Dipl. Phys. Jan Richter, Prof. Dr. Matthias Rief, Dipl. Phys. Ralf Seidel, and Dipl. Phys. Stefan Wild are gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft.

  1433-7851/00/3921-3912 $ 17.50+.50/0

base composition in order to correlate the changes in the mechanics to certain adducts. Cisplatin can form only two different adducts in p(dGdC)-p(dGdC): the intrastrand cross-link G*CG* and the interstrand cross-link between two guanine bases at any two neighboring base pairs. The untreated molecules exhibit the well known B-S transition around 70 pN and a melting transition at higher forces (Figure 2A). As both strands in p(dGdC)-p(dGdC) are also complementary to themselves, they fold back onto themselves

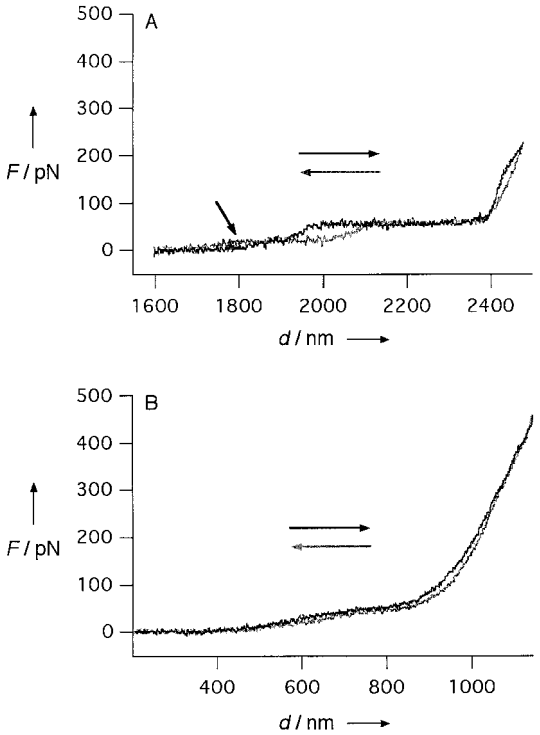

Figure 2. A) The force versus distance curves of untreated p(dGdC)-p(dGdC) shows the formation of hairpins (indicated by the arrow) after partial denaturation of the molecule. B) After reaction with cisplatin the melting is inhibited and no formation of hairpins is observed.

after initial strand separation. As reported previously, the formation of these hairpins can be observed in the force curves as plateaus at 20 pN (see arrow in Figure 2A). After platination (Figure 2B) the B-S transition loses its cooperativity and the force increases only slowly as the molecule is overstretched. The distinct melting transition has almost disappeared—there is no flattening in the curves above 80 pN. Almost no hysteresis between the stretching and relaxation traces can be found and no formation or unfolding of hairpins can be detected, even after stretching the molecules with forces of some hundred pN and keeping them stretched for a few minutes. Hence, all the structural changes that are induced in the molecules upon the application of mechanical tension are reversible on the time scale of the experiment.

Pure p(dAdT)-p(dAdT) molecules show far less mechanical stability in their native state (Figure 3A). The B-S transition starts at approximately 35 pN in the stretching curves. There is distinct hysteresis between stretching and relaxation, which shows that a force-induced melting of the duplex can already occur during the B-S transition (see also

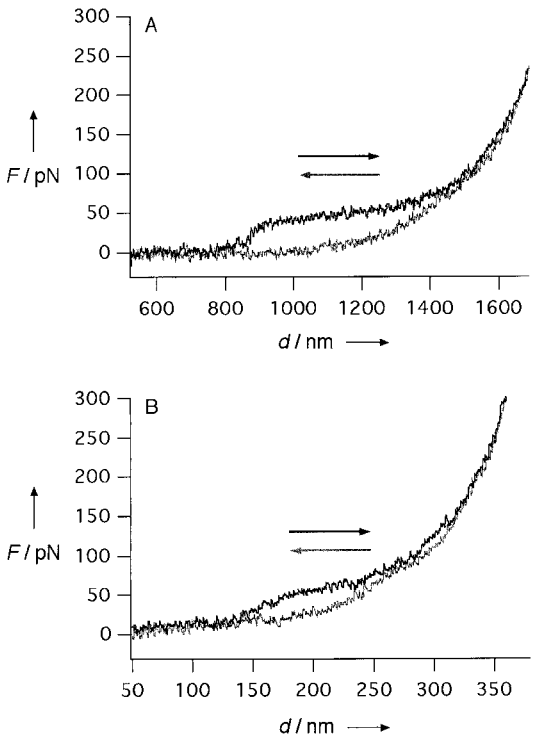

Figure 3. Force versus distance curves obtained on untreated (A) p(dAdT)-p(dAdT) and after treatment with cisplatin (B). After reaction with cisplatin the molecules can still be mechanically denatured.

[7]). No changes in the mechanical properties could be observed when p(dAdT)-p(dAdT) is stretched after incubation with cisplatin (Figure 3B). This result is not unexpected since no formation of bifunctional products of cisplatin with adenine and thymine bases has ever been reported. Hysteresis between the stretching and relaxation traces is seen, which indicates that the double helix can still be denatured by mechanical tension.

The stretching of p(dAdC)-p(dGdT), in which cisplatin can only form intrastrand cross-links between guanine bases (G*TG*), reveals more aspects of the sequence specificity of the drug. The stretching behavior of untreated molecules (Figure 4A) is very similar to that of λ-phage DNA with a cooperative B-S transition at forces of around 70 pN and melting at higher forces. In the stretching curves of the platinated molecules (Figure 4B), the B-S transition has again lost some of its cooperativity, which indicates a perturbation of the B-DNA conformation in the relaxed molecule. A second transition occurs at forces of around 300 pN which appears to be identical to the melting transition in the untreated molecules. However, upon relaxation the trace resembles the shape of the extension curve. The lack of hysteresis in the force curves, even after stretching the molecules beyond the melting transition, shows that the two strands must be able to recombine faster than the relaxation of the molecules occurs.

Cisplatin can only form intrastrand cross-links of the types G*G* or G*XG* on the p(dG) strand in p(dG)-p(dC). The stretching behavior of the native molecules (Figure 5A) is in good agreement with that of other B-DNA molecules with a cooperative B-S transition around 70 pN and a melting

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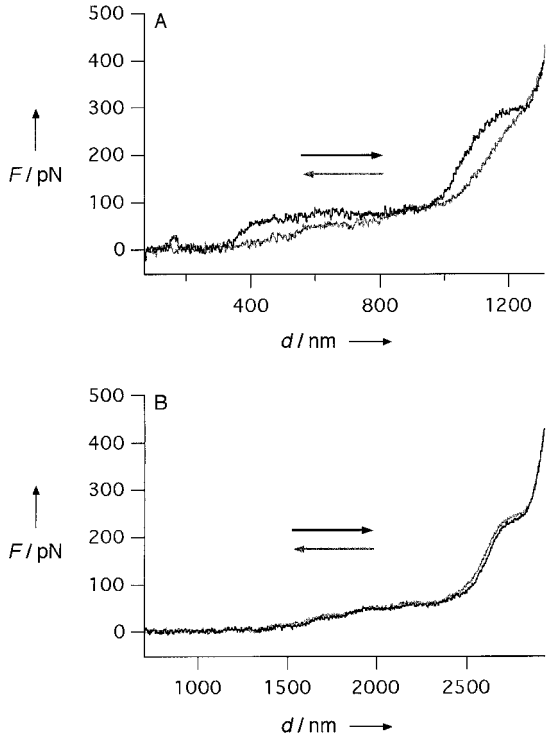

Figure 4. Force versus distance curves obtained on untreated p(dAdC)-p(dGdT) (A) and after reaction with cisplatin (B).

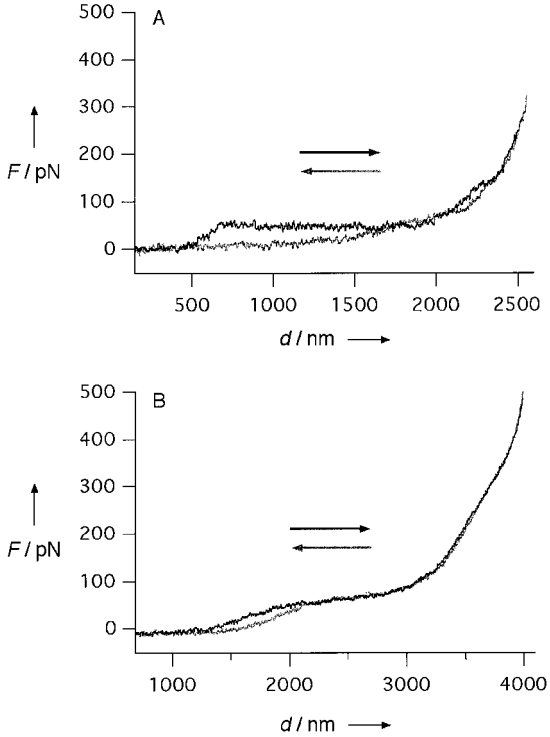

Figure 5. Force versus distance curves obtained on untreated p(dG)-p(dC) (A) and after platination (B).

transition at higher forces. The reaction with cisplatin again disrupts the B-S transition (Figure 5B), and makes it less cooperative. The melting transition has almost vanished in the stretching curves and only a slight kink above 300 pN can be seen. Compared to the untreated molecules there is only very small hysteresis between the stretching and relaxation traces. Hysteresis only occurs if the molecules are stretched with forces above 80 pN and are subsequently relaxed to about 0.7 times their contour length. This result implies the existence of a mechanically induced structural reorganization in the molecule which occurs at forces above 80 pN. However, the double helix cannot be separated permanently.

Our results show that the B-S transition is very sensitive to the binding of cisplatin, which we attribute to deviations of the molecule from the native B-DNA conformation. In all molecules in which bifunctional adducts can be formed there is a loss of cooperativity in the B-S transition, which may indicate a disruption of the cooperative units of this process. Since new theoretical models predict a strong dependence of the cooperativity of the B-S transition on the base-stacking interaction,[10] we attribute a change in the slope of the B-S plateau mainly to a change in the stacking interaction of DNA bases. Spectroscopic and calorimetric studies report lowered melting temperatures for DNA complexed with cisplatin. The reduced thermal stability is of enthalpic origin, which also suggests that the base-stacking interaction is disturbed.[8] There is no distinct melting transition in all the molecules with possible G*G* adducts or interstrand cross-links, which indicates that a loss of cooperativity occurs in the melting process too. This proposal is in agreement with the reported broader temperature range for the melting of various types of DNA after complexation with cisplatin, which also suggests a lowered cooperativity.[8] The strongly reduced hysteresis in all curves obtained for guanine containing molecules, however, suggests that—if there is strand separation in the first place—reannealing can occur much faster than in unplatinated molecules. We assume that the two strands in λ-DNA and p(dGdC)-p(dGdC) are kept in proximity to each other by the interstrand cross-links and this gives rise to a drastically accelerated reannealing of the denatured parts of the double helix which are not affected directly by the binding of the drug. The data also show that a permanent mechanical separation of the double helix is inhibited by interstrand cross-links. The intrastrand cross-links also seem to stabilize the double-stranded structure, as seen in the p(dAdC)-p(dGdT) molecules. This observation can be explained by the formation of two new hydrogen bonds in the G*TG* products. Molecular dynamics simulations show that these hydrogen bonds stabilize the double-stranded structure and keep the two strands in proximity.[11]

However, the high force transition in the platinated p(dAdC)-p(dGdT) curves shows that the intrastrand cross-links do not disrupt the force-induced melting process itself. It is likely that the undisturbed Watson–Crick base pairs in this adduct can be separated mechanically at forces similar to those in untreated molecules, which leads to the melting transition in the force versus distance curves. As there appears to be a force-induced structural change in p(dG)-p(dC) which is not observed in p(dAdC)-p(dGdT), it seems unlikely that it is caused by products of the type G*GG*. In this case further studies are needed to reveal more details about the origin of this behavior and its possible consequences.

This study demonstrates the unique potential of single-molecule force spectroscopy as an analytical tool for obtain-

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ing new structural information about DNA and its interaction with binding agents. It highlights the close correlation between structure and force and points out the particular role of force as a parameter controlling biological function, for example, in preventing mechanical strand separation in DNA.

Experimental Section

λ-BstE II digested DNA and cisplatin (*cis*-diammine(dichloro)platinum(II) were purchased from Sigma (Deisenhofen, Germany). All other DNA molecules were purchased from Pharmacia (Freiburg, Germany). For the reaction, a saturated cisplatin stock solution (50 μL) was added to a DNA-containing solution (150 μL; 100 μg mL^{-1}, 130 mM NaCl, 10 mM Tris, 1 mM EDTA, pH 8.0) and allowed to react for 24 h at 37 °C in darkness to give an excess of cisplatin per base pair. The sample preparation and the details of the force experiments are described elsewhere.[6, 7] All experiments were carried out in Tris buffer (10 mM, pH 8.0) containing NaCl (150 mM) and EDTA (1 mM). The spring constants of all cantilevers (Microlevers, Park Scientific Instruments, Sunnyvale, CA) were determined using the thermal noise method.[12] All force curves shown consist of 4096 points and were smoothed using an 11-point box integrator.

Received: February 4, 2000 [Z14648]

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Spherical Aromaticity in I_h Symmetrical Fullerenes: The $2(N+1)^2$ Rule**

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Dedicated to Professor Fred Wudl on the occasion of his 60th birthday

Aromatic compounds exhibit a significantly raised diamagnetic susceptibility.[1] The aromaticity of annulenes follows the Hückel rule. Due to their closed-shell structures, annulenes with $4N+2$ π electrons are not distorted (D_{nh} symmetry) and show strong diamagnetic ring currents, while $4N$ π annulenes are often distorted and have paratropic character. Although there is no such comparable rule for hetero- and polycyclic π systems, substructures with $4N+2$ π electrons frequently possess pronounced diamagnetic ring currents. Spherical fullerenes represent a special group of polycyclic π systems. In neutral C_{60}, an encapsulated ^{3}He nucleus is only subject to weak diamagnetic shielding.[2] The 20 six-membered rings in C_{60} show diamagnetic ring currents,[3] as indicated by the negative nucleus-independent chemical shifts (NICS)[4] in the center[5] as well as above and under the six-membered rings,[6] but this effect is roughly compensated by the 12 paratropic five-membered rings[3] (positive NICS values),[5] therefore the ^{3}He chemical shift of He@C_{60} is only $\delta = -6.3$.[2] Other neutral fullerenes,[7] such as He@C_{70}, He@C_{76}, He@C_{78}, He@C_{82}, and He@C_{84}, behave similarly[5] and the highest diamagnetic endohedral chemical shift of $\delta = -28.8$ is observed for He@C_{70}.[2] For the other cases, the chemical shifts are in the range between these two values.[5] Dramatic effects are found upon reduction to hexaanions.[8] While adding six electrons to C_{60} leads to an extreme shielding effect ($\delta(\mathrm{He@C_{60}}^{6-}) = -48.7$), the opposite effect is observed for C_{70} ($\delta(\mathrm{He@C_{70}}^{6-}) = 8.3$). Significantly, the five-membered rings in both cases become diatropic but the diatropy of the six-membered rings in C_{60} increases, whereas, to a large extent, it decreases in C_{70}. The experimentally determined ^{3}He NMR chemical shifts of fullerenes can be well reproduced computationally.[3, 5, 7, 9–13] No correlation between magnetism and cluster size or charge of fullerenes has been found as yet. Herein, we demonstrate that the total diatropy of icosahedral fullerenes such as C_{20},[14] C_{60}, and C_{80},[15] (Figure 1) and their cluster distortion depend on the degree of the electron occupation in the valence shell. The resulting $2(N+1)^2$ rule represents the spherical analog to the $4N+2$ rule for the cyclic annulenes.

The π-electron system of an icosahedral fullerene can be approximately considered as a spherical electron gas, which surrounds the surface of a sphere. The wave functions of this electron gas can be characterized by the angular momentum quantum numbers ($l = 0, 1, 2, 3, \ldots$). The s shell ($l = 0$) is

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[**] This work was supported by the Alexander von Humboldt Stiftung and the Funds der Chemischen Industrie. We thank Prof. Dr. Paul von Ragué Schleyer for valuable discussions and suggestions.