CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200500419

$[Ru(TAP)_3]^{2+}$-Photosensitized DNA Cleavage Studied by Atomic Force Microscopy and Gel Electrophoresis: A Comparative Study

Hiroshi Uji-i,[a] Philippe Foubert,[a] Frans C. De Schryver,[a] Steven De Feyter,*[a] Etienne Gicquel,[b] Ariane Etoc,[b] Cécile Moucheron,[b] and Andrée Kirsch-De Mesmaeker*[b]

Abstract: Topological modifications of plasmid DNA adsorbed on a variety of surfaces were investigated by using atomic force microscopy (AFM). On mica modified with 3-aminopropyltriethoxysilane (APS) or poly-L-lysine, the interaction between the plasmid DNA and the surface "freezes" the plasmid DNA conformation deposited from solution, and the AFM images resemble the projection of the three-dimensional conformation of the plasmid DNA in solution. Modified mica with low concentrations of Mg^{2+} leads to a decrease in the interaction strength between plasmid DNA and the substrate, and the AFM images reflect the relaxed or equilibrium conformation of the adsorbed plasmid DNA. Under these optimized deposition conditions, topological modifications of plasmid DNA were produced under irradiation in the presence of $[Ru(TAP)_3]^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene), which is a non-intercalating complex, and were followed as a function of illumination time. The observed structural changes correlate well with the conversion of the supercoiled covalently closed circular form (ccc form) into the open circular form (oc form), induced by a single-strand photocleavage. The AFM results obtained after fine-tuning of the plasmid DNA–substrate interaction compare well with those observed from gel electrophoresis, indicating that under the appropriate deposition conditions, AFM is a reliable technique to investigate irradiation-induced topological changes in plasmid DNA.

Keywords: atomic force microscopy · DNA · photolysis · ruthenium · scanning probe microscopy

Introduction

DNA photocleaving agents based on metal complexes have been developed during the last decade.[1,2] The photocleavage takes place according to a type I (by electron transfer) or type II (by singlet oxygen) oxidation mechanism. There are only a few examples of pure type I photocleavage, initiated by a photoelectron transfer from a nucleobase to a metallic photosentitizer.[3] These metallic complexes have also been examined as potential phototherapeutic drugs,[4] probes for nucleic acids, or sequence-specific photoreagents.[5] The possibility of using atomic force microscopy (AFM) to observe directly conformational transitions of plasmid DNA due to, for example, a single-strand cleavage, is very attractive because the topology of plasmid DNA plays an important role in genetic processes and in the interaction of DNA with enzymes. However, the understanding of conformational transitions of plasmid DNA as a result of a photocleavage is still rather limited. Therefore, studies at the single-biomolecule level are very useful.

AFM has been widely employed to study DNA[6] because it allows the determination of not only the averaged structural properties, but also structural heterogeneities induced by drug binding and/or photoreactions.[7,8a] The main advantages of this technique, in addition to its high spatial resolution, are the sample preparation and measuring conditions: sample preparation avoids artifacts that arise from deposition of a metal coating as used in electron microscopy, and the samples can be investigated even under buffer condi-

[a] Dr. H. Uji-i, Dr. P. Foubert, Prof. Dr. F. C. De Schryver, Dr. S. De Feyter
Department of Chemistry, Katholieke Universiteit Leuven
3001 Leuven (Belgium)
Fax: (+32) 016-327-990
E-mail: steven.defeyter@chem.kuleuven.be

[b] Dr. E. Gicquel, Dr. A. Etoc, Prof. C. Moucheron, Prof. A. Kirsch-De Mesmaeker
Organic Chemistry and Photochemistry
Université Libre de Bruxelles
CP160/08, 50 avenue F. D. Roosevelt, 1050 Brussels (Belgium)
Fax: (+32) 02-650-3018
E-mail: akirsch@ulb.ac.be

tions.[8] Hence, AFM measurements reflect more accurately the natural environment of the biomolecule.

The sample preparation for AFM, that is, deposition of the material onto a flat surface, involves the transition from three to two dimensions,[9–11] which modifies the conformation of DNA. Rivetti et al. reported that the method of immobilization is crucial for the observation of linear DNA molecules. Indeed, appropriate adhesion methods allow the adsorbed DNA molecules to obtain their equilibrium conformation even after the loss of one degree of freedom, so that they adopt the lowest energy conformation upon adsorption onto a surface.[10] However, such relaxation processes on a surface are not straightforward for plasmid DNA, because conformational transitions of plasmid DNA involve many aspects: not only inertial force and excluded volume, but also the torsional strain in the winding of plasmid DNA play a role.

Therefore, it is mandatory to investigate the effect of the transition from three to two dimensions on the appearance of plasmid DNA in the AFM images, before studying in detail the structural changes resulting from the interaction and DNA photocleavage induced by Ru^{II} complexes.[12] Photocleavage induces a conformational transition of the plasmid DNA from a supercoiled, covalently closed circular form to an open circular one.

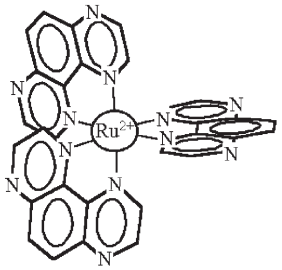

Figure 1. Chemical structure of $[Ru(TAP)_3]^{2+}$.

The Ru^{II} complex containing 1,4,5,8-tetraazaphenanthrene ligands ($[Ru(TAP)_3]^{2+}$) is a good candidate as a photosensitizer for DNA cleavage (Figure 1).[13] The π-deficient character of the TAP ligand causes the corresponding excited complexes to be more oxidizing than the excited species of most other complexes containing polypyridyl ligands, such as 2,2′-bipyridyl or 1,10-phenanthroline.[13c] It has been shown that the Ru^{II} complex with three TAP ligands is more efficient in photocleaving the DNA phosphate backbone than the other Ru^{II} polypyridyl complexes, and can form photoadducts with guanine bases. These photoreactions have been shown to be promoted by electron transfer from the guanine base of DNA to the corresponding excited complex.[13d,e] Photoinduced electron transfer can thus lead to either a photocleavage or a photoaddition. Excited $[Ru(TAP)_3]^{2+}$ produces single-strand breaks in plasmid DNA.[13] In the single-strand-breaking model, one single-strand break converts the supercoiled, covalently closed circular (ccc) form to a nicked, open circular (oc) form (Figure 2). If a cleavage occurs in the opposite strand close to the site of the initial strand cleavage, the oc form could, in principle, be converted to the linear form. These structural conversions are schematically depicted in Figure 2a. Figure 2b shows a series of AFM images of the corresponding forms.

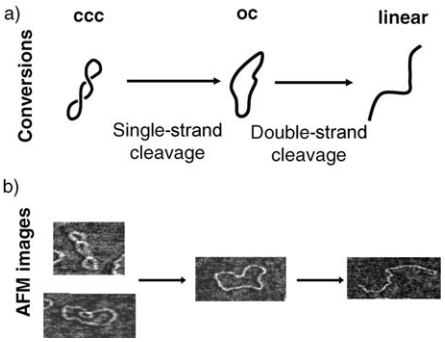

Figure 2. Photoinduced conversions of the tertiary structure of pUC18 plasmid DNA from AFM images: a) schematic drawing, b) typical AFM images of the corresponding structures.

In this study, we present the effect of immobilization strategies on the configuration of plasmid DNA adsorbed on a surface. After optimization of the deposition approach, we investigated the irradiation-induced topological changes of plasmid DNA in the presence of $[Ru(TAP)_3]^{2+}$ as a function of irradiation time. We did this by using AFM, and compared the results with those of gel electrophoresis.

Results and Discussion

To study conformational transitions of plasmid DNA as a result of photocleavage, it is important that the conformation of the plasmid DNA as imaged on a surface reflects its intrinsic conformation. However, during deposition of plasmid DNA onto a surface, the conformation is inevitably modified by the transition from three to two dimensions. It has been reported that the conformational flexibility of long polymers on a surface strongly depends on interactions with this surface.[9–11] Therefore, we initially examined different immobilization strategies, that is, treatment of the mica surface with Mg^{2+}, 3-aminopropylethoxy silane (APS), and poly-L-lysine. These methods provide different adhesion forces; Mg^{2+}-mica[14] at a relatively low concentration of Mg^{2+} and APS-mica,[15] which was prepared from diluted APS solution, provide weak immobilization conditions. Poly-L-lysine-coated mica is considered to provide stronger immobilization, due to the high density of adhesion sites, which is promoted by amino groups.

Figure 3a–c show typical AFM images of *non-irradiated* pUC18 plasmid DNA in the presence of $[Ru(TAP)_3]^{2+}$ on mica modified with Mg^{2+} (5 mM in water), APS (0.01 wt% in water), and poly-L-lysine (0.01 wt% in water), respectively. The DNA solution before deposition contains ~80% of the ccc form and ~20% of the oc form, as determined by performing gel electrophoresis. Figure 3a shows that on Mg^{2+}-mica, both the ccc and oc forms can be clearly identified. Under these conditions, the ratio of ccc form:oc form was the same as that observed in solution, as determined from more than ten locations on two samples prepared from the same solution. This is not the case for surfaces that have

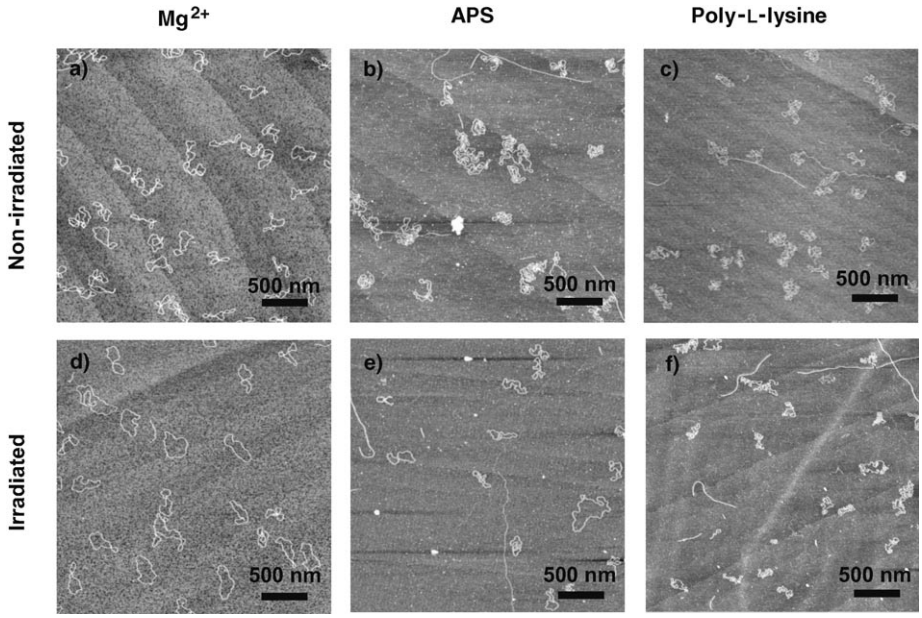

Figure 3. AFM images of pUC18 plasmid DNA in the presence of $[Ru(TAP)_3]^{2+}$ deposited on mica: a)–c) from non-irradiated solution, d)–f) from irradiated solution. The mica surface was pretreated with Mg^{2+} (a,d), APS (b,e), or poly-L-lysine (c,f).

been treated differently. Thus, on both APS- (Figure 3b) and poly-L-lysine-coated mica (Figure 3c), plasmid DNA adopts a more tightly coiled structure. On these substrates, the ccc form was the dominant conformation (>90%). In addition, aggregates are sometimes observed on APS- and poly-L-lysine-coated mica, which prevents the identification of individual plasmid DNA strands. Note that the linear chains observed on APS- and poly-L-lysine-coated mica are features of the surface itself and do not originate from the linear DNA form. AFM images of plasmid DNA obtained in the absence of $[Ru(TAP)_3]^{2+}$ show the same results (data not shown), confirming that, because of its non-intercalating binding mode, $[Ru(TAP)_3]^{2+}$ does not induce significant morphological changes in plasmid DNA.

Figure 3d–f show AFM images of *irradiated* plasmid DNA in the presence of $[Ru(TAP)_3]^{2+}$ on mica treated with Mg^{2+}, APS, and poly-L-lysine, respectively. Note that the solution was irradiated before deposition on the substrate. By using agarose gel electrophoresis, it was estimated that the same irradiated solution contained 38% of the ccc and 62% of the oc form. On Mg^{2+}-mica (Figure 3d), the conformation of the plasmid DNA clearly changed from a supercoiled structure to a relaxed form, compared to non-irradiated DNA, as shown in Figure 3a. From the AFM images, the relative amounts of the ccc and the oc form were estimated to be ~60 and 40%, respectively, which is in good agreement with the gel electrophoresis measurements. The plasmid DNA on APS-mica shown in Figure 3e displays more coiled structures (>80%) than is seen on Mg^{2+}-mica, although the former exhibit more relaxed forms than the non-irradiated plasmid DNA on APS-mica (Figure 3b). On poly-L-lysine-modified mica, less difference is observed between irradiated and non-irradiated samples.

The different results obtained from the various surfaces can be related to the different immobilization strategies, in combination with the salt effect ($MgCl_2$) on the DNA conformation. During deposition of plasmid DNA from solution onto the surface, the apparent conformation could change. For example, the oc form of plasmid DNA appears as a coiled-like conformation on poly-L-lysine, which makes it difficult to distinguish between the ccc and oc forms in two-dimensional images.[9,10] The strong adhesion on poly-L-lysine does not allow the plasmid DNA to relax into its intrinsic equilibrium conformation after adsorption, with the effect that most of the plasmid DNA might appear to have a coiled configuration. Thus, the immobilization energy is too great to allow the observation of any change due to irradiation. In contrast, with low concentrations of Mg^{2+}- and APS-mica, the equilibration due to the relatively weak adhesion might allow the plasmid DNA to remain relaxed in its intrinsic conformation after adsorption. Although on APS-mica, which was prepared from a dilute solution of APS, the relaxed form could be observed, the number of plasmid DNA strands per AFM image was very low, and the reproducibility of the statistical analysis was inferior to the results obtained with Mg^{2+}-mica. Therefore, we chose Mg^{2+}-modified mica to study the DNA photocleavage in more detail.

In addition to the deposition process, we must also take into account the effect of ionic strength on DNA conformation[11,14] and the interaction of Ru complexes with DNA.[13] To investigate the ionic strength effect and to select the ideal concentration of Mg^{2+}, we determined the relative occurrence of the different plasmid DNA forms on mica as a function of the concentration of Mg^{2+} (Figure 4). The solution was prepared as before and the same irradiation conditions as those described for the investigation of the effect of the immobilization methods were used. In the presence of a high concentration of Mg^{2+} (37 mM), approximately 70% of the plasmid DNA appears to have a ccc-like configuration on the mica surface after illumination, which is much higher than the proportion in solution determined by electrophoresis. As the concentration of Mg^{2+} was reduced, the relative amount of the ccc-like configurations decreased. At less than 5 mM Mg^{2+}, the percentage of ccc and oc configurations was similar to that obtained with gel electrophoresis.

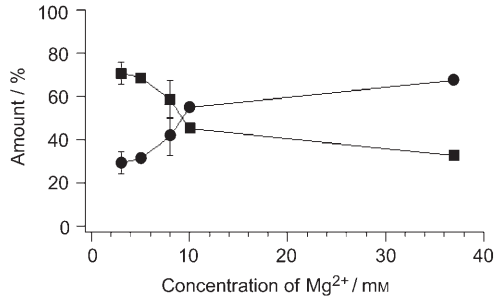

Figure 4. Dependence upon [Mg^{2+}] of the deposition of plasmid DNA on a mica surface, shown as the proportion of the ccc-like (●) and oc-like (■) forms as a function of Mg^{2+} concentration. The solution was irradiated with 0.4 W cm^{-2} for 70 s at 458 nm.

Therefore, we used a Mg^{2+} concentration of 3–5 mM for the experiments described below. At such relatively low concentrations of Mg^{2+}, Mg^{2+}-mica provides the highest reproducibility for statistical analyses of AFM images and the best agreement with the results of gel electrophoresis, although the presence of $MgCl_2$ can have an unwinding/winding effect on plasmid DNA in solution before deposition. However, this excellent agreement between the results of the two experimental techniques strongly suggests that at the low $MgCl_2$ concentration used, salt-induced winding/unwinding effects do not play a crucial role, and that adsorption occurs under equilibrium conditions, as predicted by some earlier reports.[8–11]

After the selection of the substrate and optimization of the deposition conditions, a kinetic study of the photocleavage was conducted by performing gel electrophoresis and AFM. The results of a typical DNA photocleavage experiment at [bp]/[Ru] = 20 are presented in Figure 5 (bp = base pairs). The filled and open symbols in Figure 5 show the relative amounts of the ccc and oc plasmid DNA forms as a function of irradiation time, identified by AFM and agarose gel electrophoresis, respectively. Before irradiation, the solution contained approximately 82% of the ccc and 18% of the oc form. The non-irradiated stock DNA solution without metal complexes exhibited the same ratio. The amount of the oc form increases at the expense of the ccc form as irradiation time increases. After 15 s of irradiation, only 33% of the ccc form was observed and 67% of the oc form was already present. As shown in Figure 5, the product profiles for AFM and gel electrophoresis are similar: in both cases, the photoconversion reaches a plateau after 15 s of irradiation. The differences between both analyses are less than 10% at the low concentration of Mg^{2+} employed in this study. The fact that a plateau is reached after a certain illumination time has already been observed,[16] and would be due to photodechelation of the starting complex and/or formation of photoadducts of the metallic species to DNA, two processes that consume the photosensitizer.

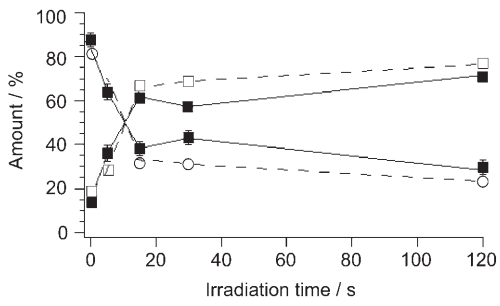

Figure 5. The relative amounts of the ccc (● and ○) and oc (■ and □) forms as a function of irradiation time. The filled and open symbols indicate the results of AFM and gel electrophoresis, respectively. 5 mM of Mg^{2+} was used in the preparation of the AFM samples.

Conclusion

We have investigated the effect of different DNA immobilization strategies on mica for the study of conformational transitions of plasmid DNA as a result of photoinduced cleavage by [Ru(TAP)$_3$]$^{2+}$. We compared the effect of the substrate on the immobilization of plasmid DNA. Mg^{2+}-mica at low Mg^{2+} concentrations provides the weakest adhesion force and supports equilibration conditions, which allows plasmid DNA to relax, so that its intrinsic conformation in solution is reflected upon adsorption onto the surface. As well as the nature of the substrate, the Mg^{2+} ratio plays a role in the case of Mg^{2+}-mica. Only at low concentrations is there good agreement between the results of AFM and gel electrophoresis experiments regarding the relative amounts of the ccc and oc forms. These results are promising in view of future investigations by means of AFM of the structural conversion from the ccc to the oc form. The deposition conditions and analysis described here will facilitate the study of DNA conformational changes as a result of photocleavage at the single-molecule level, as well as in situ investigations in water.

Experimental Section

Sample preparation: Ru(TAP)$_3$Cl$_2$ was synthesized and purified according to a previous procedure.[13a] The pUC18 plasmid DNA from *Escherichia coli* pRI, with 2686 base pairs (bp), was purchased from Sigma-Aldrich. The stock solution of the plasmid DNA was prepared in 10 mM Tris buffer and stored at −20 °C. 3-Aminopropylethoxy silane (APS) and poly-L-lysine were purchased from Sigma-Aldrich and were used without further purification. Water was obtained from a Millipore Milli-Q purification system (18 MΩ). The concentration of Ru(TAP)$_3^{2+}$ in the aqueous solutions was determined by using absorption spectroscopy with a molar extinction coefficient of $\varepsilon_{437} = 13000\ M^{-1}cm^{-1}$.[13d]

The photolysis solutions at molar ratio [bp]/[Ru] = 20 were prepared by mixing the DNA stock solution and Ru(TAP)$_3^{2+}$ solution and leaving them to equilibrate for 15 min. The plasmid DNA solutions were photolysed by irradiation at 458 nm (Ar-ion laser, Stabilite 2017, Spectra-Physics). The excitation light was expanded and collimated by two lenses to irradiate the complete sample volume. Samples contained 25 μL of ruthenium complex (1.1 μM) and pUC18 ([bp] = 22 μM) so that only a small fraction of light was absorbed during photolysis. 20 μL of the irradiated solution was analyzed by performing gel electrophoresis and another 5 μL was diluted with tris buffer and used for AFM measurements. All

operations with DNA–Ru complexes were conducted under dimmed-light conditions.

DNA adhesion onto Mg^{2+}-mica: The non-irradiated and irradiated solutions were diluted to 1 $\mu g mL^{-1}$ of plasmid DNA in tris-HCl (10 mM) and $MgCl_2$ (3–5 mM). The Mg^{2+} ions were added to promote DNA adhesion to a mica surface.[17] Note that $MgCl_2$ was added after illumination. This prevents, due to electrostatic interaction of Mg^{2+} with DNA, the replacement of metal complexes by Mg ions from plasmid DNA.[13b] The solution (8–10 µl) was deposited on freshly cleaved mica (~8×8 mm^2). After 2 min of incubation at room temperature, the samples were gently rinsed with 5×2 mL of Milli-Q water and blown dry with clean compressed Ar gas before AFM measurements were taken.

DNA adhesion onto mica modified with APS and poly-L-lysine: The pure APS solution was diluted with Milli-Q water to a concentration of 0.01–0.001 % by volume. 10–15 µL of the solution was deposited onto freshly cleaved mica. After 20 min of incubation, the sample was rinsed with 5 mL Milli-Q water to remove excess APS solution and then dried with Ar gas.[15]

10 µL of 0.01 wt % poly-L-lysine aqueous solution was deposited onto freshly cleaved mica and incubated for 1 min. Then the substrate was rinsed with 5 mL Milli-Q water and dried with Ar gas.[18]

For both modified mica substrates, 10 µL of the plasmid DNA solution (1 $\mu g mL^{-1}$) was deposited on the substrate and incubated for 2 min. Then the sample was rinsed with Milli-Q water and dried with Ar gas.

Gel electrophoresis analysis: Irradiated samples were loaded on a 1 % agarose horizontal gel (SubCell GT, BioRad) and migration was performed for 2–3 h by using a TAE buffer with the voltage set at 70 V. The gel was then stained with an ethidium bromide (EtBr) solution and DNA bands were visualized by using a UV transilluminator equipped with a CCD camera. Quantification of the various plasmid DNA forms was realized by using the Quantity One 4.2 software from BioRad. A corrective factor of 1.66 was used for the ccc form, due to the lower accessibility to the EtBr of the supercoiled form. In some gel electrophoresis data of irradiated pUC18 plasmid DNA in the presence of $[Ru(TAP)_3]^{2+}$, a band that increased in intensity as a function of irradiation time was observed. This suggested the presence of a linear DNA form, even before irradiation. It was subsequently found that this spot was due to the presence of a DNA impurity in the sample.[19]

AFM imaging and analysis: AFM measurements were performed by using either a Multimode AFM with a Nanoscope IV controller (Veeco/Digital Instruments, Santa Barbara, USA) or a PicoSPM (Molecular Imaging, Arizona, USA) with the Acoustic AC-module (Molecular Imaging, Arizona, USA) and SPM-1000 control unit (RHK Technology, Michigan, USA). Samples were imaged in air in tapping mode with a drive frequency of 200–300 kHz. Silicon nitride oxide-sharpened tips (NCHR, Nanosensors, Germany) were used. All images were background leveled (up to second order).

According to the model (Figure 2), the relative amount of different plasmid DNA forms was statistically evaluated for approximately 500 plasmid DNAs in more than ten AFM images, collected on two samples that were prepared from the same solution. Reliable analysis requires the collection of more than five images at different positions for each sample, typically one at the center and four around the center. This is because the population of each form was heterogeneously distributed on the mica substrate. Only those plasmid DNAs that were completely visible within the frame of an AFM image were used for the analysis. In the AFM images presented here, the height of the DNA was measured to be ~0.5 nm, as expected from previous reports. Note that all DNA samples on mica were kept in moisture-free conditions before AFM measurements were made.

Acknowledgements

The authors thank the DWTC, through IUAP-V-03, and the Institute for the Promotion of Innovation by Sciences and Technology in Flanders (IWT). H.U. thanks the KU Leuven for a grant in the framework of a Interdisciplinary Research Programme (IDO). S.D.F. is a postdoctoral fellow of the Fund for Scientific Research-Flanders. The "Action de Recherche Concertée" (ARC-programme 02/07–286) is gratefully acknowledged for the financial support and a postdoc fellowship for E.G.

[1] B. Norden, P. Lincoln, B. Akerman, E. Tuite in *Metal Ions in Biological Systems, Vol. 33* (Eds.: A. Sigel, H. Sigel), Marcel Dekker, **1996**, pp. 177–252.
[2] E. D. Stemp, J. K. Barton in *Metal Ions in Biological Systems, Vol. 33* (Eds.: A. Sigel, H. Sigel), Marcel Dekker, **1996**, pp. 325–365.
[3] C. Moucheron, A. Kirsch-De Mesmaeker, J. Kelly in *Structure and Bonding, Vol. 92* (Ed.: M. J. Clarke), Springer, **1998**, pp. 163–216.
[4] R. Blasius, C. Moucheron, A. Kirsch-De Mesmaeker, *Eur. J. Inorg. Chem.* **2004**, 3971–3979.
[5] O. Lentzen, E. Defrancq, J.-F. Constant, S. Schumm, D. Garcia-Fresnadillo, C. Moucheron, P. Dumy, A. Kirsch-De Mesmaeker, *J. Biol. Inorg. Chem.* **2004**, *9*, 100–108.
[6] a) H. G. Hansma, *J. Vac. Sci. Technol. B* **1996**, *14*, 1390–1394; b) H. G. Hansma, I. Revenko, K. Kim, D. E. Laney, *Nucleic Acids Res.* **1996**, *24*, 713–720.
[7] a) S. D. Jett, D. I. Cherny, V. Subramaniam, T. M. Jovin, *J. Mol. Biol.* **2000**, *299*, 585–592; b) M. Lysetska, A. Knoll, D. Boehringer, T. Hey, G. Krauss, G. Krasch, *Nucleic Acids Res.* **2002**, *30*, 2686–2691; c) J. E. Coury, L. McFail-Isom, L. D. Williams, L. A. Bottomley, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12283–12286; d) J. E. Coury, J. R. Anderson, L. McFail-Isom, L. D. Williams, L. A. Bottomley, *J. Am. Chem. Soc.* **1997**, *119*, 3792–3796; e) T. Berge, N. S. Jenkins, R. B. Hopkirk, M. J. Waring, J. M. Edwardson, R. M. Henderson, *Nucleic Acids Res.* **2002**, *30*, 2980–2986.
[8] a) L. H. Pope, M. C. Davies, C. A. Laughton, C. J. Roberts, S. J. B. Tendler, P. M. Williams, *Anal. Chim. Acta* **1999**, *400*, 27–32; b) H. G. Hansma, J. Vesenka, C. Siegerist, G. Kelderman, H. Morrett, R. L. Sinsheimer, V. Elings, C. Bustamante, P. K. Hansma, *Science* **1992**, *256*, 1180–1184; c) F. Nagami, G. Zuccheri, B. Samorì, R. Kuroda, *Anal. Biochem.* **2002**, *300*, 170–176.
[9] D. Lang, P. Coates, *J. Mol. Biol.* **1968**, *36*, 137–151.
[10] a) C. Rivetti, M. Guthold, C. Bustamante, *J. Mol. Biol.* **1996**, *264*, 919–932; b) C. Rivetti, C. Walker, C. Bustamante, *J. Mol. Biol.* **1998**, *280*, 41–59.
[11] M. Bussiek, N. Mücke, J. Langowski, *Nucleic Acids Res.* **2003**, *31*, e137.
[12] E. Yavin, E. D. A. Stemp, L. Weiner, I. Sagi, R. Arad-Yellin, A. Shanzer, *J. Inorg. Biochem.* **2004**, *98*, 1750–1756.
[13] a) A. Kirsch-De Mesmaeker, R. Nasielski-Hinkens, D. Maetens, P. Pauwels, J. Nasielski, *Inorg. Chem.* **1984**, *23*, 377–379; b) J. M. Kelly, D. J. McConnell, C. OhUigin, A. B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein, J. Nasielski, *J. Chem. Soc. Chem. Commum.* **1987**, 1821–1823; c) A. B. Tossi, J. M. Kelly, *Photochem. Photobiol.* **1989**, *49*, 545–556; d) J. P. Lecomte, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **1995**, *34*, 6481–6491; e) J. P. Lecomte, A. Kirsch-De Mesmaeker, J. M. Kelly, A. B. Tossi, H. Görner, *Photochem. Photobiol.* **1992**, *55*, 681–689; f) A. Del Guerzo, A. Kirsch-De Mesmaeker, *Inorg. Chem.* **2002**, *41*, 938–945.
[14] a) D. Lang, H. Bujard, B. Wolff, D. Russell, *J. Mol. Biol.* **1967**, *23*, 163–181; b) D. I. Cherny, T. M. Jovin, *J. Mol. Biol.* **2001**, *313*, 295–307.
[15] K. Umemura, M. Ishikawa, R. Kuroda, *Anal. Biochem.* **2001**, *290*, 232–237.
[16] P. Vicendo, S. Mouysset, N. Paillous, *Photochem. Photobiol.* **1997**, *65*, 647–655.
[17] H. G. Hansma, D. E. Laney, *Biophys. J.* **1996**, *70*, 1933–1939.
[18] J. Hu, M. Wang, H.-U. Weier, P. Frantz, W. Kolbe, D. F. Ogletree, M. Salmeron, *Langmuir* **1996**, *12*, 1697–1700.
[19] A control plasmid DNA (pUC19 DNA, New England BioLabs) did not exhibit any linear form.

Received: April 15, 2005
Revised: July 15, 2005
Published online: October 14, 2005

