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Study of the modifications caused by cisplatin, transplatin, and Pd(II) and Pt(II) mepirizole derivatives on pBR322 DNA by atomic force microscopy

G.B. Onoa, V. Moreno *

Departament de Química Inorgànica, Universitat de Barcelona, Avgda Diagonal 647, 08028 Barcelona, Spain

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Abstract

Modifications of the structure of pBR322 DNA caused by interaction with cisplatin, transplatin and Pd(II) and Pt(II) mepirizole derivatives were studied. The compounds were incubated with the plasmid DNA for 24 h at 37 °C and then observed with an atomic force microscope. Circular DNA was used because the small tertiary structural changes are easy to monitor. Likewise, its superhelical nature mimics DNA better than certain forms of intracellular DNA such as chromatin. AFM images clearly reveal that the complexes induce changes in the topological forms of fully relaxed pBR322 DNA. Most of the compounds produce a more compact DNA structure with modified writhing number. Analysis of gel migration of the relaxed pBR322 DNA incubated with the platinum complexes provides complementary information, which is in good agreement with AFM results.

Keywords: AFM DNA-metal complexes images; Pd(II) and Pt(II) mepirizole complexes; pBR322 DNA modifications

1. Introduction

Mepirizole is an active pyrimidino-pyrazole derivative used as an analgesic and anti-inflammatory agent. In recent years, considerable attention has been paid to pyrazoles, pyrimidines and related N-containing heterocyclic derivatives. Systems of this kind play a significant role in many biological processes, due to their coordinating ability for a trace metal ion. Furthermore, the use of mepirizole as a ligand has an advantage since its biological toxicity is low (Takabatake et al., 1970). Pd(II) and Pt(II) complexes with mepirizole were synthesized and characterized (Onoa et al., 1999). Two isostructural complexes (Fig. 1) were obtained and studied by different techniques. The interaction of the complexes with linear DNA (*Calf thymus*) was followed by CD. Antiproliferative studies of both Pd and Pt complexes on six different cell tumor lines were carried out (Onoa et al., 1999).

DNA molecule is the most important intracellular target for antitumour Pt drugs (Crul et al., 1997; Lepre and Lippard, 1990; Pil and Lippard, 1997). Therefore, a good parameter to predict

* Corresponding author. Tel.: +34-93-402-1914; fax: +34-93-490-7725

E-mail address: virtudes.moreno@qi.ub.es (V. Moreno).

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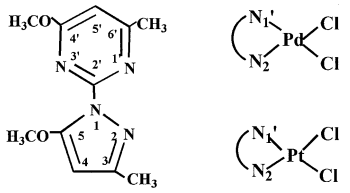

Fig. 1. Schematic molecular representation of mepirizole and its Pd(II) and Pt(II) complexes.

whether these compounds can act as anti-cancer agents it is to study their reactivity with the DNA. Changes in the tertiary structure can be detected by electrophoretic and microscopy studies. Tertiary structure is a significant factor in a number of genetic functions. Thus, DNA supercoiling has been extensively studied because its tertiary structure is related to the biological activity of DNA. Topological structures of pBR322 DNA have widely been studied by different techniques: electrophoresis, CsCl/ethidium bromide gradient, electron microscopy among others (Huang and Chen, 1990; Samorí et al., 1993a; Samorí et al., 1993b; Samorí et al., 1996; Zhang et al., 1996; Fritzsche et al., 1996; Wang et al., 1996; Tian et al., 1997). Modifications of superhelical density and winding of pBR322 by cisplatin have also been studied (Bouayadi et al., 1992; Ushay et al., 1981). Several topological structures of pBR322 DNA observed by AFM in contact mode and under air (Zhang et al., 1996; Fritzsche et al., 1996; Wang et al., 1996) as well as under liquid (Samorí et al., 1993a; Samorí et al., 1993b; Samorí et al., 1996), with and without previous treatments respectively, have been reported. Samorí (Samorí et al., 1993a; Samorí et al., 1993b; Samorí et al., 1996) and Fritzsche (Fritzsche et al., 1996) observed that non-pretreated pBR322 DNA molecules look like plectonemic superhelices with a specific local chirality. Samorí (Samorí et al., 1993a; Samorí et al., 1993b; Samorí et al., 1996) reported that UV irradiation of plasmid DNA induced a nick and thus gradual relaxation in the circular plasmid DNA. However Bai (Zhang et al., 1996; Wang et al., 1996; Tian et al., 1997) reported five different DNA topological structures using pBR322 from E. Coli HB101.

In the present study the interaction of cisplatin, transplatin and the Pd(II) and Pt(II) mepirizole complexes with pBR322 plasmid DNA has been studied by electrophoretic mobility and atomic force microscopy (AFM).

2. Experimental

2.1. Materials and methods

The complexes were prepared as described (Onoa et al., 1999) using $K_2[PdCl_4]$ and $K_2[PtCl_4]$ from Johnson Matthey (Reading, UK). Cisplatin and transplatin were purchased from Johnson Matthey. Mepirizole, Tris, Tris–HCl, HEPES, ethidium bromide and boric acid were from ICN (Barcelona, Spain) and used without further purification. The solvents were from Fluka (Madrid, Spain), CT-DNA, EDTA and BSA from Sigma (Madrid, Spain), pBR322 and DTT from Boehringer Mannheim (Germany). Ultrapure agarose was obtained from ECOGEN (Barcelona, Spain); $MgCl_2$ and KCl from Merck (Darmstad, Germany)

2.2. Gel electrophoresis of drug–pBR322 Complexes

pBR322 DNA aliquots (0.25 μg/mL) were incubated in the presence of the compounds in TE buffer at several input molar rations (ri = 0.05, 0.10,0.30 and 0.50) for electrophoresis studies. Incubation was carried out in the dark at 37 °C for 24h; 24mL aliquots of complex-DNA compounds containing 0.5 μg/DNA underwent 1% agarose gel electrophoresis for 4h at 2 V/cm in 0.5XTBE (45mM Tris–borate, 1mM EDTA pH = 8.0) buffer. Gel was subsequently stained in the same buffer containing ethidium bromide (1 mg/mL). The gel was photographed with an image Master® VDS, Pharmacia Biotech.

2.3. Atomic force microscopy

2.3.1. Preparation of DNA

In order to optimize the observation of the conformational changes in the tertiary structure of

pBR322 DNA plasmid, the negative superhelical strain was relieved by previous relaxation with DNA topoisomerase I (topo I) (Life Technologies. Gibco BRL, Gaithersburg MD). This enzyme breaks and rejoins one strand of duplex DNA. pBR322 DNA plasmid (5 μg) was incubated with 10 U of DNA topo I (1U is the amount of enzyme required to relax 0.5 μg of plasmid DNA) in 50 mM Tris–HCl pH = 7.5, 120 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM dithiotritol and 30 mg/mL BSA in a final volume of 200 μL. After incubation at 37 °C for 7 h, the mixture was extracted twice with phenol and once with chloroform at 25 °C. The aqueous phase was recovered and DNA was precipitated twice with 2.5 volumes of cold ethanol and 0.1 volume of 3M NaAcO, pH = 4.8. The DNA was washed in 70% ethanol and suspended in 100 mL of sterile Milli-Q water. The degree of supercoiling of the substrate was monitored by electrophoresis on 1% agarose gel in the same conditions as above. A similar procedure was described by Bouayadi (Bouayadi et al., 1992) to evaluate the evolution of cisplatin –DNA monoadducts into biadducts depending upon superhelical density.

2.3.2. Preparation of adducts DNA–metal complexes

15 ng of pBR322 DNA was incubated in an appropriate volume with the required platinum concentration corresponding to the molar ratio ri = 0.5. Cisplatin, transplatin and mepirizole complexes were dissolved in HEPES buffer (4mM HEPES pH = 7.4, 5mM KCl and 2mM $MgCl_2$) but metal–mepirizole complexes were dissolved in a DMSO-HEPES (30:70) mixture, since they are only slightly soluble in HEPES buffer. The different solutions as well as Milli-Q water were passed through 0.2 nm FP030/3 filters (Scheicher & Schueell GmbH, Germany) and centrifuged at 4000 g several times to avoid salt deposits and provide a clear background when they were imaged by AFM. The reactions were run at 37 °C for 24 h in the dark. After the incubation, the DNA–metal complexes were precipitated twice with ethanol, washed in 70% ethanol and then suspended in 30mL of HEPES buffer. In order to verify the purity of the adducts formed, as well as the structural changes occurring in the DNA, the ligated complexes were electrophoresed on 1.5% agarose gel for 4h at 2 V/cm.

2.3.3. Sample preparation for atomic force microscopy

Samples were prepared by placing a drop (6 μL) of DNA solution or DNA–metal complex solution onto Ni-treated green mica (Ashville-Schoonmaker Mica Co., Newport New, VA). The Ni-treated mica was prepared according to the literature (Macquet and Butour, 1978; Mong et al., 1981). After adsorption for five min at room temperature, the samples were rinsed for 10s in a jet of deionized water of 18 $MW cm^{-1}$ from a Milli-Q water purification system directed onto the surface with a squeeze bottle. They were then placed into ethanol–water mixture (1:1) five times, plunged three times each in ethanol 100%. The samples were blow dried with compressed argon over silica gel and then imaged in the AFM.

2.3.4. Imaging by atomic force microscopy

The samples were imaged in a Nanoscope III Multimode AFM (Digital Instrumentals Inc., Santa Barbara, CA) operating in tapping mode in air at a scan rate of 1–3 Hz. The AFM probes was 125 mm-long monocrystalline silicon cantilever with integrated conical shaped Si tips (Nanosensors GmbH Germany) with an average resonance frequency $f_o = 330$ KHz and spring constant K = 50 N/m. The cantilever is rectangular and the tip radius given by the supplier is 10 nm, a cone angle of 35° and high aspect ratio. In general, the images were obtained at room temperature ($T = 23 \pm 2°C$) and the relative humidity (RH) was typically 55%.

Apparent contour lengths of the plasmids were obtained using digital analysis software (IMAT, Serveis Científico-Tècnics of the University of Barcelona, Spain). Mean apparent heights and mean apparent width of DNA were measured using cross-sections of the images in the Nanoscope software, command of Nanoscope III version 4.1. The heights were measured from top of the molecule relative to an average background level and the widths were measured at the half height of a molecule. Four different samples of

each reaction were imaged in several places and many times in order to obtain reliable measurements.

3. Results and discussion

3.1. Electrophoretic mobility study

The influence of the compounds on the tertiary structure of DNA was determined by its ability to modify the electrophoretic mobility of the covalently closed circular (ccc) and open (oc) forms of pBR322 plasmid DNA. Fig. 2 shows the mobility of the native pBR322 plasmid DNA, of the plasmid DNA incubated with cisplatin as positive control, of free mepirizole and its Pd (II) and Pt(II) complexes at different concentrations. The behaviour of the gel electrophoretic mobility of both forms, ccc and oc, of pBR322 plasmid (Fig. 2, slot 1) and DNA:cisplatin adducts (Fig. 2, slots 2–4) are consistent with previous reports (Ushay et al., 1981). Metal-free mepirizole did not alter the electrophoretic mobility of the ccc or oc forms of the DNA at any concentration (Fig. 2, slots 5 to 8). However, the mobility of the oc form is increased by incubation with higher concentration of Pd-mep (Fig. 2, slots 9 to 12). The increase caused by incubation with Pt-mep in the oc form is only moderate while the mobility of the ccc band form decreases slightly (Fig. 2, slots 13–16).

The changes in the electrophoretic mobility of both ccc and oc forms of pBR322 DNA after incubation with the metal complexes suggest that the Pt-complex binding is analogous to that of the cisplatin, although less drastic. However, the Pd-complex only seems to affect the oc form. Since the increase in the mobility of the oc form caused by Pd-mep was greater than that caused by Pt-mep at equivalent metal:nucleotide ratio it can be deduced that the relaxed form of pBR322 has been compacted or that the palladium complex has introduced conformational changes which modify the degree of superhelicity of the DNA molecules. In contrast, the free ligand does not seem to modify the tertiary structure of DNA.

3.2. Atomic force microscopy

Since gel electrophoresis showed that metal complexes had a greater effect on the tertiary structure of the oc form than on the supercoiled form, the relationship between DNA compacting and the superhelical degree was studied by direct visualization of various relaxed circular DNA species using Tapping Mode Atomic Force Microscopy (TMAFM). Changes in the tertiary structure of the DNA have been studied extensively

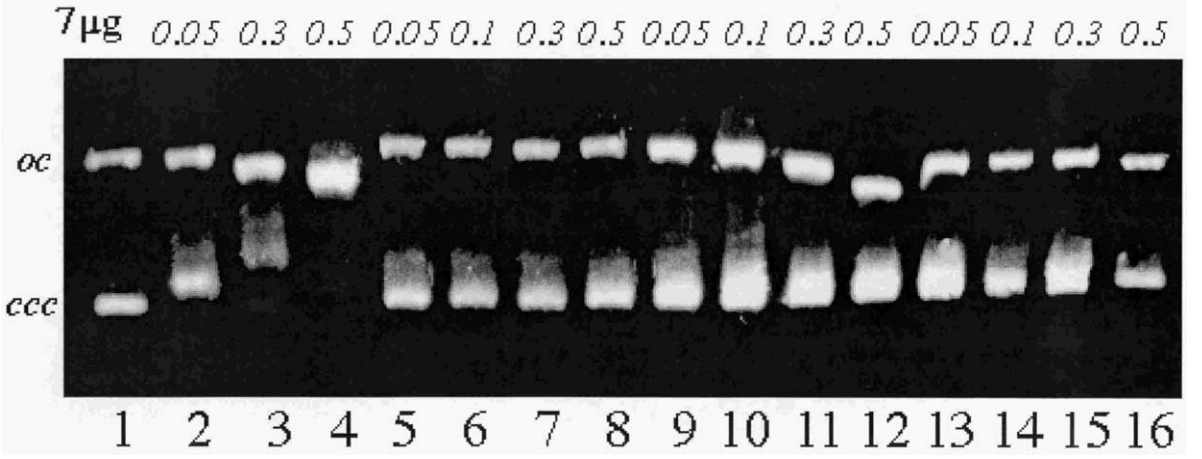

Fig. 2. Changes in the electrophoretic mobility of pBR322 plasmid DNA after incubation with cisplatin, mepirizole and mepirizole Pd(II) and Pt(II) complexes. Lane 1, pBR322; lanes 2–4, cisplatin; lanes 5–8, mepirizole; lanes 9–12, Pd-mepirizole complex; lanes 13–16, Pt-mepirizole complex. (OC, open circular form; CCC covalently closed circular form).

because they are linked to its biological activity. It is a significant factor in a number of genetic functions.

To facilitate observation of topological changes after incubation with the drugs, we used pBR322 fully relaxed by topo I (Bouayadi et al., 1992; Foglesong and Reckord, 1992; Keller, 1975). Topoisomerases modify the topology of DNA (Wang, 1996).

The tertiary structure of DNA is affected by environmental conditions such as ionic strength, temperature and pH (Wang et al., 1996; Lyubchenko and Shlyakhtenko, 1997; Thomson et al., 1996). We worked in conditions (neutral pH and low ionic strength) in which it has been shown that circular DNA retains its intrinsic geometric properties in order to ensure that subsequent changes are caused by the complexes.

A typical large-scale AFM image of native pBR322 without any treatment is shown in Fig. 3A. High resolution images of a well-known plectonemic superhelix of DNA molecule obtained by scanning over a small area are shown in Fig. 3A (see insert). The main feature of the native samples prepared as described above is that DNA molecules have the irregular geometry reported for typical supercoiled pBR322 DNA molecules (Samorí et al., 1993b; Samorí et al., 1996; Zhang et al., 1996; Fritzsche et al., 1996; Boles et al., 1990): rosette-like rather than plectonemic superhelical form, or assymmetric figure of eight. The mean apparent heights and widths are presented in Table 1 and Fig. 4; they are consistent with those reported earlier (Fritzsche et al., 1996). The lengths could not be measured owing to the great interaction between the molecules.

Fig. 3B shows an overview of naked pBR322 DNA relaxed with topo I. Most of them are relaxed circular DNA homogeneously distributed on the mica surface; they look like ring structures with different diameters. The geometry of the molecules is reminiscent of the AFM images of complexes of circular DNA-ReCa protein reported by Lyubchenko et al. (Lyubchenko et al., 1995) and those of pBR322 obtained from E. Coli HB101 by Bai (Zhang et al., 1996). In contrast, the geometry is different from that of pUC18 plasmid relaxed by topo I, reported by Jovin (Schaper et al., 1993) or that of plasmids that had been exposed to UV radiation in order to include nicks and subsequently relaxation (Samorí et al., 1996; Fritzsche et al., 1996). In Fig. 3B there are some ring-like structures connected by two or more molecules in a few areas; they are aggregated into oligomeric catenates. Grain-like species or supercoiled and supercondensed DNA were not detected in our case as a consequence of the previous relaxing process. In addition, the background was very clear.

Significant differences between Fig. 3A and Fig. 3B can be detected; they are consistent with those observed by electrophoresis (Fig. 5). In both AFM and electrophoresis approximately 80% of the molecules are fully relaxed after topoisomerase treatment. AFM images indicate that about 87% of relaxed circular pBR322 DNA are monomers, about 10.3% of them are dimers and the remaining 2.7% are connected to oligomers. Relaxed molecules are more firmly attached to the mica than native molecules. Furthermore, relaxed molecules appeared to have a specific orientation whereas native molecules are heterogeneously distributed on the mica. This fact suggests that after digestion with topo I, the orientation of the DNA phosphate groups changes which leads to a special distribution of the molecules over the mica. It is also interesting to compare the degree of DNA superhelicity in these two images.

The twisting number (Tw) is a sensitive function of experimental conditions and it is related to the writhing number (Wr), which is a geometric property of the DNA axis. Wr is directly proportional to the number of times that DNA winds round the supercoiling axis and it is equal to zero for any closed curve lying on a sphere or a plane. The shape of DNA rings is shown in Fig. 3B. The insert shows a 10x magnification of one ring. We therefore conclude that the value of Wr of the relaxed pBR322 DNA molecules with topo I under our conditions is close to zero, while supercoiled pBR322 presents a different value (Fig. 3A).

The apparent contour lengths of pBR322 DNA molecules from four different samples with scan sizes of 10 mm x10 mm were measured. Statistics results are presented in Fig. 4. The results indicate that 69.5% of the monomeric molecules have a

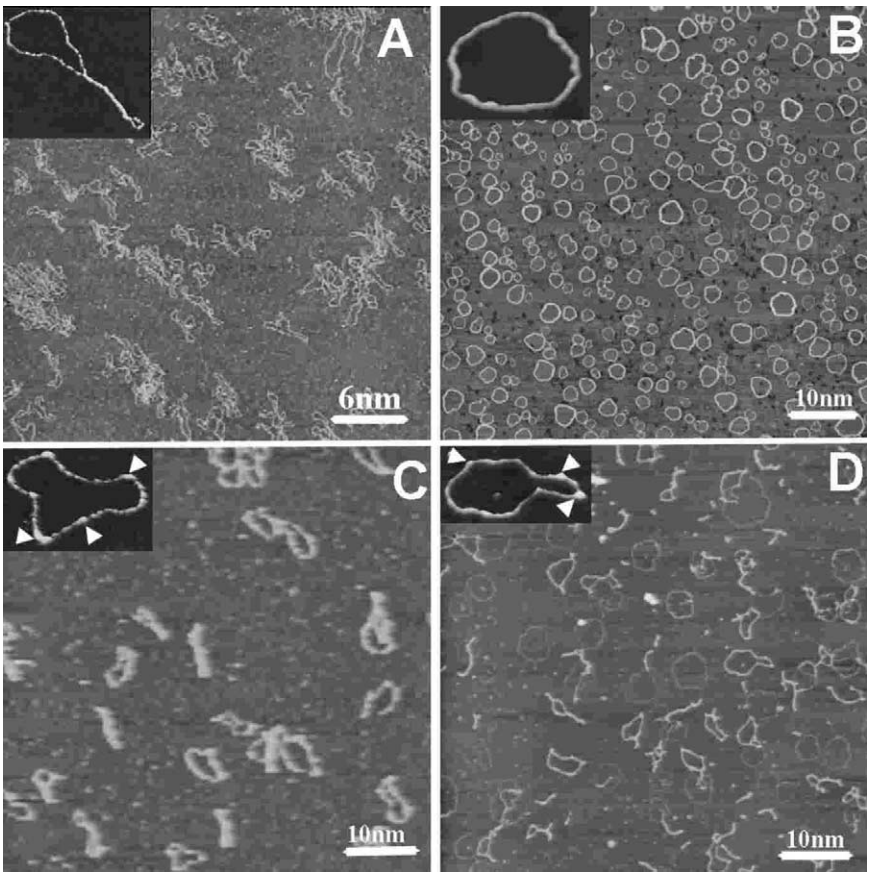

Fig. 3. TMAFM images in dry of pBR322 plasmid DNA in HEPES buffer at concentration of 0.5 ng/mL adsorbed on Ni-treated mica. (A) native pBR322 without any treatment; (B) pBR322 DNA relaxed with DNA topoisomerase I; (C) and (D) relaxed pBR322 DNA incubated with cis- and trans- platin respectively (ri = 0.5) at 37°C for 24 h. The inserts are a three-dimensional enlargement of a small scale image; the headarrows indicate bright blobs attached to DNA molecules. The sizes are 5mm × 5mm and the inserts are 400nm × 400nm.

Table 1
Dimensions of the molecules obtained from TMAFM

Compounds	Length (mm)	Height (nm)	Width (nm)
Native DNA	–	0.62 ± 0.19	19.32 ± 2.9
Relaxed DNA	1.45 ± 0.53	1.19 ± 0.37	27.83 ± 4.5
Cisplatin:DNA	1.09 ± 0.22	0.94 ± 0.6	84.1 ± 31.2
Transplatin:DNA	1.12 ± 0.5	0.58 ± 0.3	37.17 ± 8.7
mep:DNA	1.42 ± 0.29	1.12 ± 0.21	22.89 ± 4.4
Pd-mep:DNA	1.22 ± 0.44	1.97 ± 0.35	83.40 ± 19.3
Pt-mep:DNA	1.34 ± 0.17	0.4 ± 0.16	22.26 ± 9.1

mean apparent length of 1.45 mm. These measurements correspond to an average of 0.33 nm per base; it is the value corresponding to the B form of DNA, which is according with the reported in the literature (Fritzsche et al., 1996). 18.2% of the molecules have a diameter of less than 1 mm, corresponding to an average of 0.21 nm per base; a similar result was recently reported by Bai. for pBR322 DNA (Tian et al., 1997). Some relaxed DNA molecules were probably dehydrated by buffer treatment and exposure to ethanol during the preparation for AFM as reported elsewhere (Hansma et al., 1992; Hansma et al., 1993). There is also evidence that cations such as Na^+ or Mg^{2+} reduce the effective diameter of DNA molecules (Cheng and Bremer, 1997). An alternative explanation could be that double stranded DNA, when

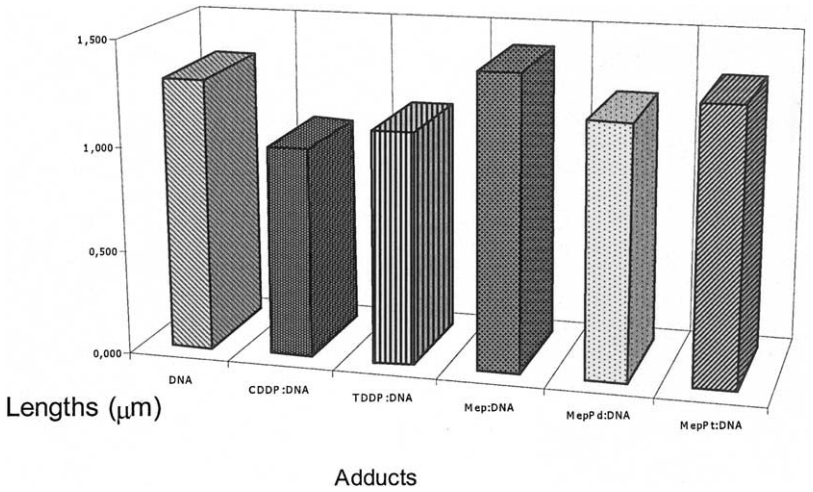

Fig. 4. Quantitative analysis of TMAFM images. Histograms of the mean apparent contour lengths measured from pBR322 plasmid DNA. Comparison between different samples imaged.

it is constrained to a mica surface, may be pulled into a different conformation from typical A or B forms (Hansma et al., 1992). The mean heights and widths are reported in Table 1. The widths are consistent with the expected values because of convolution during imaging with tips that have radii of curvature between 10–20 nm (Bustamante et al., 1993; Bustamante et al., 1994). The results are consistent with those reported for typical pBR322 DNA (Fritzsche et al., 1996; Tian et al., 1997).

Several microscopy studies of DNA incubated with cisplatin have been reported in the last two decades (Cohen et al., 1979; Macquet and Butour, 1978; Mong et al., 1981; Revet et al., 1984; Jeffrey et al., 1993; Rampino, 1992; Onoa et al., 1998); all of them have demonstrated that cisplatin and transplatin shorten DNA. Besides, platinum binding to pBR322 DNA after 24 h is 80% and the mobility of the oc band increases slightly over time (Ushay et al., 1981). The results obtained here with pBR322 are consistent with those reported earlier. Fig. 3C and 3D show that full relaxation of pBR322 is lost after the incubation with cisplatin and transplatin respectively. Most molecules are newly supercoiled upon platinum binding and several geometries are also observed. Most of the supercoiled molecules have branched interwound shapes (Fig. 3C). Unbranched supercoils with extended shapes, fully interwound or locally unwound into loops of variable size are also observed (Fig. 3D). Several uncoiled molecules show kinks and bends in twisted-circle configuration, which suggest transition states from relaxed to supercoiled forms. Many circular DNA molecules contain one or more intersections characteristic of DNA superhelical turns. These images suggest that after incubation with cisplatin and transplatin Wr number is non zero, as found for naked DNA; however, strong shortening is not evident. The mean apparent lengths, widths and heights are compared in Fig. 4 and Table 1. All these results are consistent with those given by electrophoresis Fig. 6A. DNA increases in mobility upon platinum binding; it migrates in smear bands indicating shortened and partially collapsed structures, which could be associated with changes in superhelical density. Small bright areas signifying blob-like feature along the DNA molecules are also seen; some examples are indicated by arrowheads in enlarged images (inserts).

AFM images were also obtained for relaxed pBR322 incubated with mepirizole and Pd- and Pt-mepirizole. In Fig. 7A, pBR322 incubated with

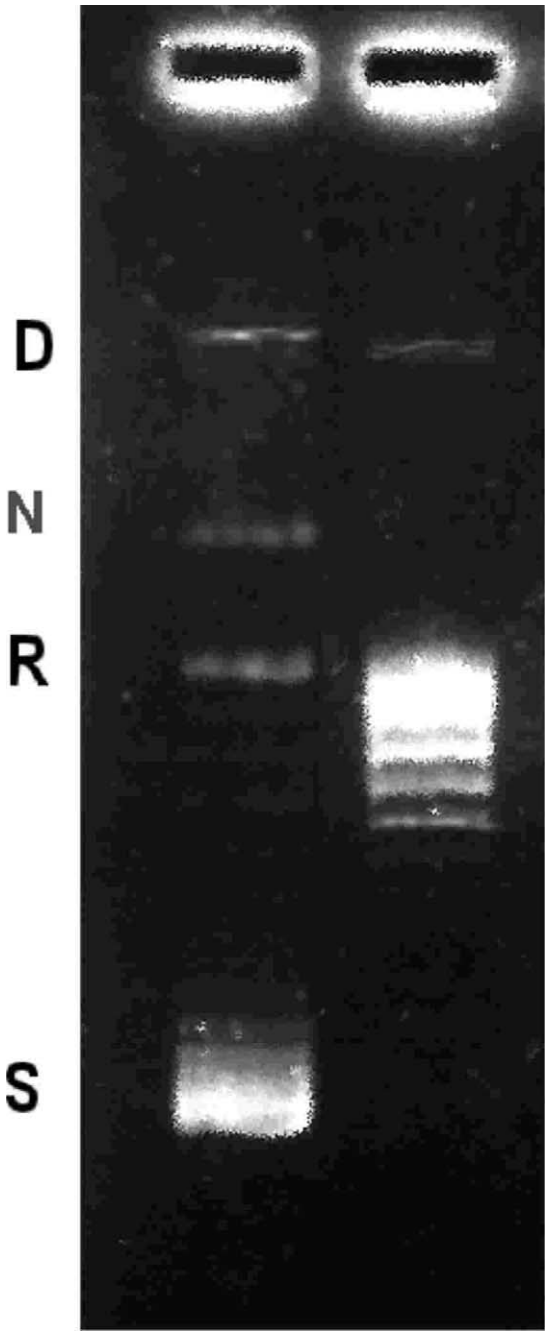

Fig. 5. Agarose gel electrophoresis of pBR322 DNA relaxed by Calf Thymus DNA Topoisomerase I. 5 μg of DNA were reacted with 10U of DNA topo I as described in materials and methods. Dimeric, nicked, relaxed and supercoiled DNA are labeled D, N, R, and S respectively.

mepirizole at 37 °C for 24 h is shown. Outstanding changes in the geometry or dimensions of the molecules are not observed, as expected. The mean apparent lengths, widths and heights are shown in Fig. 4, Table 1. DNA molecules show slight damage, due probably to changes in imaging forces. Few differences can be observed with regard to free DNA; there are no differences between the diameters of the molecules; the orientation in diagonal direction has been lost; the absorption of the molecules on the mica is apparently not as efficient as in the case of free DNA. All these features suggest that mepirizole induces a slight modification on the conformation of DNA bases or phosphate groups.

In the case of DNA incubated with Pd-mep noticeable changes can be see (Fig. 7B). The DNA molecules are collapsed; this effect is similar to the cluster of Bluescript plasmid observed by Hansma (Hansma et al., 1993) in HEPES solution, or the supercondensed pBR322 DNA reported by Bai (Zhang et al., 1996). A magnified molecule is shown in the insert; the collapsed form is evident. A spectacular increase in the height and the width is noticed as quoted in Table 1. We reported a similar effect of derivatives of palladium on DNA molecules (Onoa et al., 1998). Some blobs are also observed along the molecule (see insert). The AFM image of Pt-mep:DNA complex in Fig. 7C also shows that the platinum complex modified the writhing number of relaxed plasmid in a similar way to cisplatin although the effect is much less drastic. A few 'beads-on-string' filaments are formed (see insert), which are 1.5 times wider and 2.5 times higher than the corresponding bare regions of DNA. Mean apparent lengths, widths and heights are compared in Table 1 and Fig. 4. We speculate that these brighter beads could correspond to the bound metallic complexes in the same way as *cis*- and *trans*platin, although higher resolution studies are neccessary. A curious feature of these images is that their background is covered with small balls. This might correspond to possible aggregations of the platinum complexes. As in the above cases, AFM and electrophoresis results are consistent (Fig. 6B).

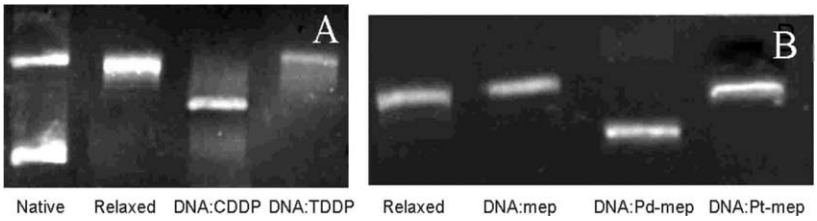

Fig. 6. Agarose gel of relaxed pBR322 DNA incubated with the compounds. (A) comparison between native DNA (slot 1), relaxed DNA (slot 2) and relaxed DNA incubated with cisplatin (slot 3) and transplatin (slot 4). (B) relaxed DNA (slot 1), relaxed DNA incubated with mepirizole (slot 2), with Pd-mepirizole (slot 3) and Pt-mepirizole (slot 4). The incubations were carried out as described in Materials and methods.

4. Conclusions

We show that AFM can provide valuable information on the interaction of certain drugs with DNA. Both palladium and platinum mepirizole complexes can interact with DNA plasmid. They can modify the tertiary structure of pBR322, especially the relaxed form. The results were compared with the modifications induced by *cis* platin and *trans* platin. The palladium derivative produced stronger changes in DNA than did other molecules.

Significant changes in the topological form of fully-relaxed pBR322 DNA plasmid can be observed by atomic force microscopy. Changes in the superhelical degree and in the writhing number of the circular DNA are easily detected. The comparison of AFM results with those obtained by electrophoresis show that they are complementary and consistent with each other. Besides, the technique can be used to study DNA topology following treatments with DNA topoisomerases.

We believe that AFM provides significant complementary information about the activity of certain platinum complexes on DNA and we are convinced that improvements in the conditions of the experiments and in the image resolution could reveal specific information about local modifications.

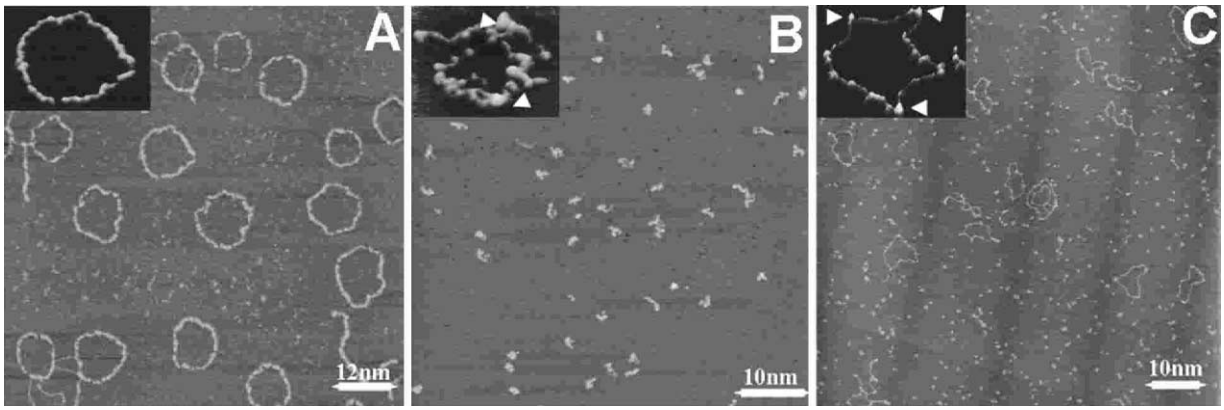

Fig. 7. Selection of TMAFM images from different runs of relaxed pBR322 plasmid DNA incubated with mepirizole and metal–mepirizole complexes at 37 °C for 24 h and a molar ratio of 0.5. Comparison of (A) relaxed pBR322 DNA complexed with mepirizole, image size 2mm × 2mm; (B) relaxed pBR322 complexed with Pd-mepirizole; (C) relaxed DNA complexed with Pt-mepirizole. The inserts are a three-dimesional enlargement of a small scale image; the headarrows indicate bright blobs attached to DNA molecules. The B and C sizes are 5mm × 5mm and the inserts are 400nm × 400nm

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