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Structural changes of linear DNA molecules induced by cisplatin

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ABSTRACT

Interaction between long DNA molecules and activated cisplatin is believed to be crucial to anticancer activity. However, the exact structural changes of long DNA molecules induced by cisplatin are still not very clear. In this study, structural changes of long linear double-stranded DNA (dsDNA) and short single-stranded DNA (ssDNA) induced by activated cisplatin have been investigated by atomic force microscopy (AFM). The results indicated that long DNA molecules gradually formed network structures, beads-on-string structures and their large aggregates. Electrostatic and coordination interactions were considered as the main driving forces producing these novel structures. An interesting finding in this study is the beads-on-string structures. Moreover, it is worth noting that the beads-on-string structures were linked into the networks, which can be ascribed to the strong DNA–DNA interactions. This study expands our knowledge of the interactions between DNA molecules and cisplatin.

1. Introduction

cis-Diamminedichloroplatinum(II) (cisplatin), one of most widely used anticancer drugs, has been applied to treat a variety of tumors, especially testicular, ovarian, head and neck, bladder and small cell lung cancers [1]. It is widely accepted that the most important target for cisplatin is DNA molecules. Cisplatin can bind on DNA molecules in 1,2-intrastrand and interstrand modes [2–4]. Recent studies demonstrated that 1,2-intrastrand cross-links were the most important configurations for cytotoxicity [1]. The major intrastrand adducts are the 1,2-intrastrand cross-links at the d(GpG) and d(ApG) sites [5]. Significant progress has been made in the past to elucidate the three dimensional structures of these 1,2-intrastrand cross-links. X-ray diffraction studies of the crystal structure of 1,2-intrastrand cross-links revealed that the DNA helix is bent toward the major groove and unwound, though the bending angles and unwinding degree vary between studies [1,2]. The NMR solution structure of a DNA dodecamer duplex containing a cis-Diammineplatinum(II) d(GpG) intrastrand cross-link revealed greater bend angles than those observed in the X-ray crystal structure [6,7]. Despite the recent progress achieved in this field, high resolution structures of 1,2-intrastrand cross-links obtained so far are usually restricted to oligodeoxynucleotide samples containing one d(GpG) target. It is necessary to investigate the interactions of cisplatin with long DNA molecules which contain multiple targets since nuclear DNA *in vivo* is a long molecule.

In addition to the NMR and X-ray diffraction techniques, atomic force microscopy (AFM) has been utilized to observe the interactions of cisplatin and DNA molecules. One advantage of AFM study is that structural changes of long dsDNA molecules induced by cisplatin can be monitored. Recently, some interesting DNA structures after platinum anticancer drug binding have been characterized by AFM [8–12]. It was revealed that the DNA structural changes induced by cisplatin are complex, influenced by the concentration and the initial state of cisplatin, the length of the DNA fragment and the reaction time [11].

In this study, we further explore the interactions of long linear DNA molecules and activated cisplatin. A novel beads-on-string structure has been observed and a possible formation process for these novel structures is proposed.

2. Materials and methods

Long linear pUC118 Pst1/BAP DNA (3162 bp) and oligodeoxynucleotide 5′-AGAGAGAGAG-3′ [$(AG)_5$] were purchased from TaKaRa Biotechnology (Dalian, China) Co., Ltd. Cisplatin was purchased from Kunming Guiyan Pharmaceutical Co., Ltd.

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2.1. Preparation of AFM sample

Before incubation with DNA, cisplatin was activated with $AgNO_3$ solution [6,13]. Cisplatin was converted to the diaqueous derivative by reacting with two equivalents of $AgNO_3$ in aqueous solution at room temperature in the dark. Then the mixture was centrifuged at 12,000 rpm for 10 min twice to remove AgCl precipitation. The interactions of DNA and cisplatin were studied by mixing equivalent volumes and then incubating for a predefined time at 37 °C in the dark. The final solution was used for AFM sample preparation. The mixed solution was dropped on a freshly cleaved highly ordered pyrolytic graphite (HOPG) surface. After incubation for 5 min, the HOPG surface was washed with pure water five times and then dried by argon gas before imaging.

2.2. Atomic force microscopy

A PicoPlus II AFM system from Molecular Imaging Inc. was used. The scanner was calibrated by a standard reference consisting of 2 μm pitch lines with a depth of 200 nm provided by Molecular Imaging Inc. All AFM images were obtained at 512 × 512 resolution in tapping mode in air at ambient conditions at a scan rate of 1.0 lines/s. A NSC35-type ultra-sharp silicon cantilever (from Mikro-Masch, Estonia) with a nominal force constant of 4.5 N m^{-1} and nominal radius of curvature less than 10 nm was used. The cantilever was oscillated at a nominal resonance of approximately 170 kHz and scan rate of 1 lines/s. The height was measured manually with PicoScan 5.3.3 software. The heights of DNA were determined from the difference between the highest point of these objects and the HOPG surface. The height results of DNA structures are averaged from at least 50 different positions in the AFM images.

3. Results and discussion

Fig. 1 shows the representative AFM topographic image of pUC118 Pst I/BAP DNA (2 ng/μl) in the absence of cisplatin on HOPG surface. Individual pUC118 DNA molecules can be differentiated by AFM and they appeared as a well-extended chain. The average

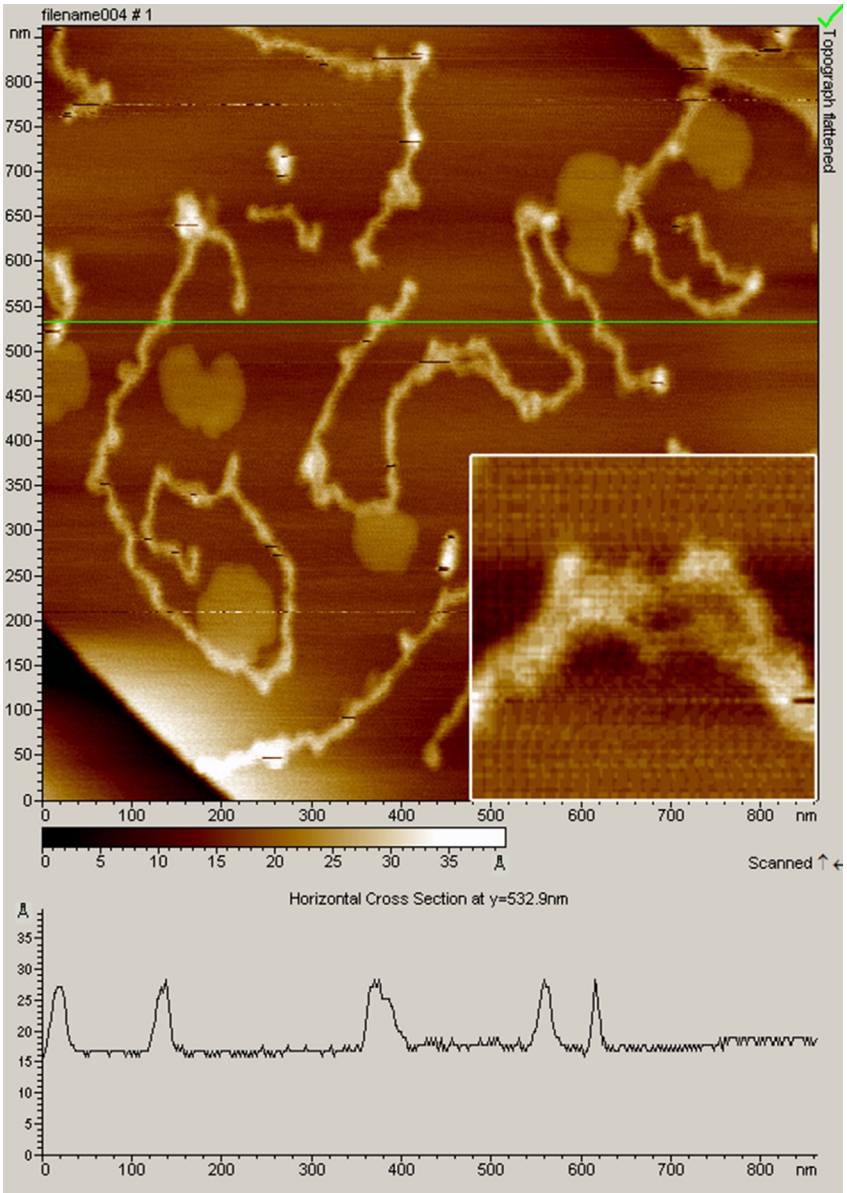

Fig. 1. The representative AFM topographic image of pUC118 Pst I/BAP (2 ng/μl) on HOPG surface.

height of individual DNA molecules was 1.14 ± 0.41 nm and average length was 1.24 ± 0.11 μm. The measured height of DNA is lower than the theoretical height of double-stranded DNA (~2.0 nm). Compression of the molecule by the tip during the scanning process and shrinking of DNA after drying process are the probable reasons for this reduction. The measured length of linear pUC118 DNA is longer than that in B-form (calculated by 0.34 nm/bp as for B-form DNA; the length of pUC118 DNA in B-form should be 1.08 μm). The increased length can be ascribed to the partial denaturation of pUC118 DNA molecules on HOPG surface. The inset in Fig. 1 shows the segment of one pUC118 DNA molecule denatured into two separated chains. The phenomena is consistent with our previous report [14]. Notably, most of the pUC118 DNA molecules separated from each other in the absence of cisplatin.

Fig. 2 shows the typical AFM topographic image of pUC118 Pst I/BAP DNA (2 ng/μl) on HOPG surface after incubation with 83 μmol/l cisplatin for 15 min, 4 h and 6 h, respectively. After incubation with activated cisplatin for 15 min, pUC118 DNA entangled into a complex network along with some unusual nodes as shown in Fig. 2a. Unexpectedly, pUC118 DNA became uniform beads-on-string structures after 4 h incubation as shown in Fig. 2b. The inset in Fig. 2b shows a high resolution AFM image of these beads-on-string structure. Average height of these beads was 3.97 ± 0.95 nm. The calculated horizontal diameter of these beads after deconvolution by a previously proposed formula is around 6 nm [15]. To our knowledge, these uniform beads-on-string structures induced by cisplatin have not been well-characterized previously. As the incubation time increased to 6 h, in addition to the beads-on-string structures, some large aggregates were formed as shown in Fig. 2c. In comparison with pUC118 DNA structure in the absence of cisplatin, the structural changes of pUC118 DNA in the presence of cisplatin were very significant with increased reaction time.

At the initial stage, the activated cisplatin can coordinate with the N7 of guanine or adenine on DNA to form the monoadduct. The activated cisplatin is $[Pt(NH_3)_2]^{2+}$,which is positively charged. When bound on DNA molecules, the interactions between the different DNA molecules should be enhanced through electrostatic attraction, which facilitates formation of a network structure as indicated in Fig. 2a. As the incubation time increases, the $[Pt(NH_3)_2]^{2+}$ cation can further coordinate with N7 of the adjacent guanine or adenine to form a di-adduct. The formation of *cis*-$[Pt(NH_3)_2d(GpG-N7(1),N7(2))]$ and *cis*-$[Pt(NH_3)_2d(ApG-N7(1),N7(2))]$ are believed to be crucial to cytotoxicity. There are more than 130 adjacent GG and 160 adjacent AG sequences distributed on one chain of pUC118 DNA (GenBank: E14304.1), which are the targets for $[Pt(NH_3)_2]^{2+}$ to form 1,2-intrastrand d(GpG) and d(ApG) adducts. NMR and X-ray crystal observations of a duplex dodecamer reveal that formation of 1,2-intrastrand d(GpG) can significantly unwind and bend DNA [2,6]. For instance, NMR determination of solution structure indicated that formation of *cis*-$[Pt(NH_3)_2d(GpG-N7(1),N7(2))]$ causes the adjacent guanine bases to roll toward one another by 49°, leading to an overall helix bend angle of 78° [6]. If this result is still valid for long linear DNA such as pUC118 DNA, many kinks should result from accumulated bending of hundreds of intrastrand adducts. Thus, the observed beads-on-string is believed to represent kinked DNA. The measured dimensions of these beads support this hypothesis. The average height of these beads in AFM images correspond to the sum height of three to five DNA double strands, consistent with a DNA kink. The large aggregates found after 6 h of incubation (Fig. 2c), can be ascribed to the further crosslinking of these beads by $[Pt(NH_3)_2]^{2+}$.

In order to confirm the observed phenomena, we further observed the interactions of single stranded oligonucleotides and cisplatin by AFM to verify these novel structures. Fig. 3 shows the typical AFM topographic image of oligodeoxynucleotide AGAGAGAGAG (abbrev. as $(AG)_5$) and their assembly structures on HOPG surface after incubation with 83 μmol/l cisplatin for 30 min and 1 h. Interestingly, oligonucleotide $(AG)_5$ assembled into a parallel pattern in the absence of cisplatin. It is believed that van der Waals and electrostatic interactions play an important role in the formation of these parallel patterns. After incubation with activated cisplatin for 30 min, oligonucleotide $(AG)_5$ appeared as a short worm-like structure as illustrated in Fig. 3b. After 1 h incubation, $(AG)_5$ further changed into the interconnected particles shown in Fig. 3c. All these results revealed that structural changes of oligonucleotide induced by activated cisplatin are similar to those induced in long linear DNA. The short worm-like structure and the

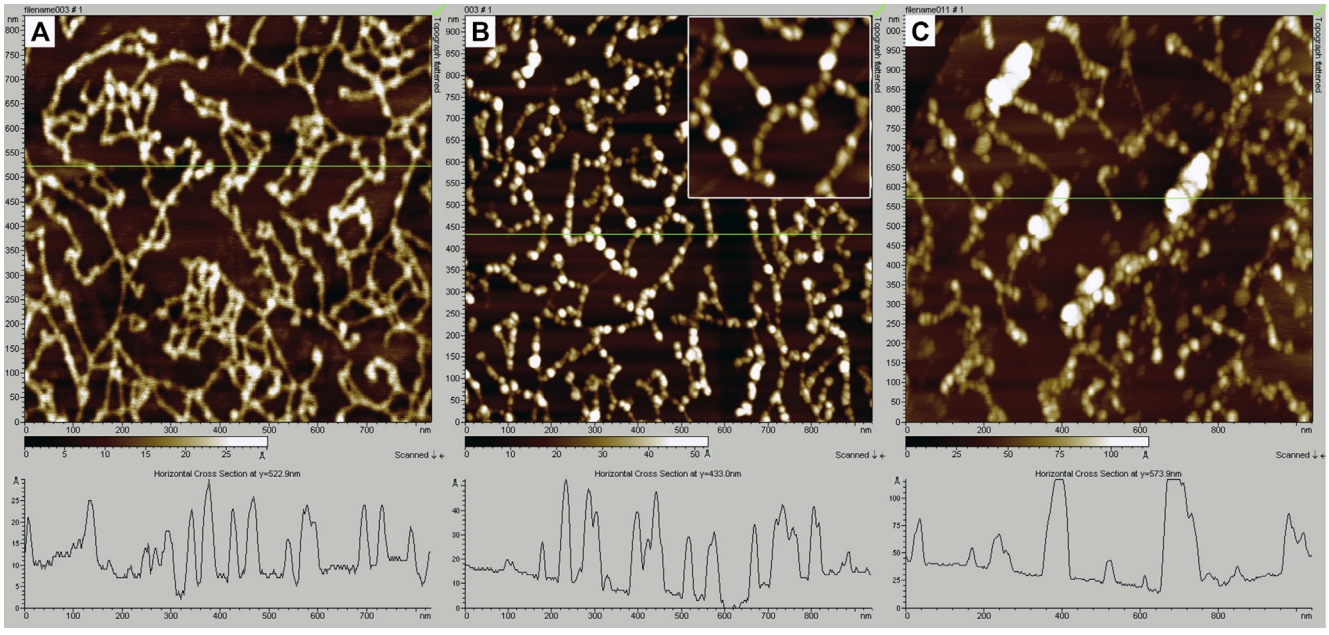

Fig. 2. The typical AFM topographic image of pUC118 Pst I/BAP(2 ng/μl) on HOPG surface after incubation with 83 μmol/l cisplatin for the predefined time. (a) 15 min (b) 4 h (c) 6 h.

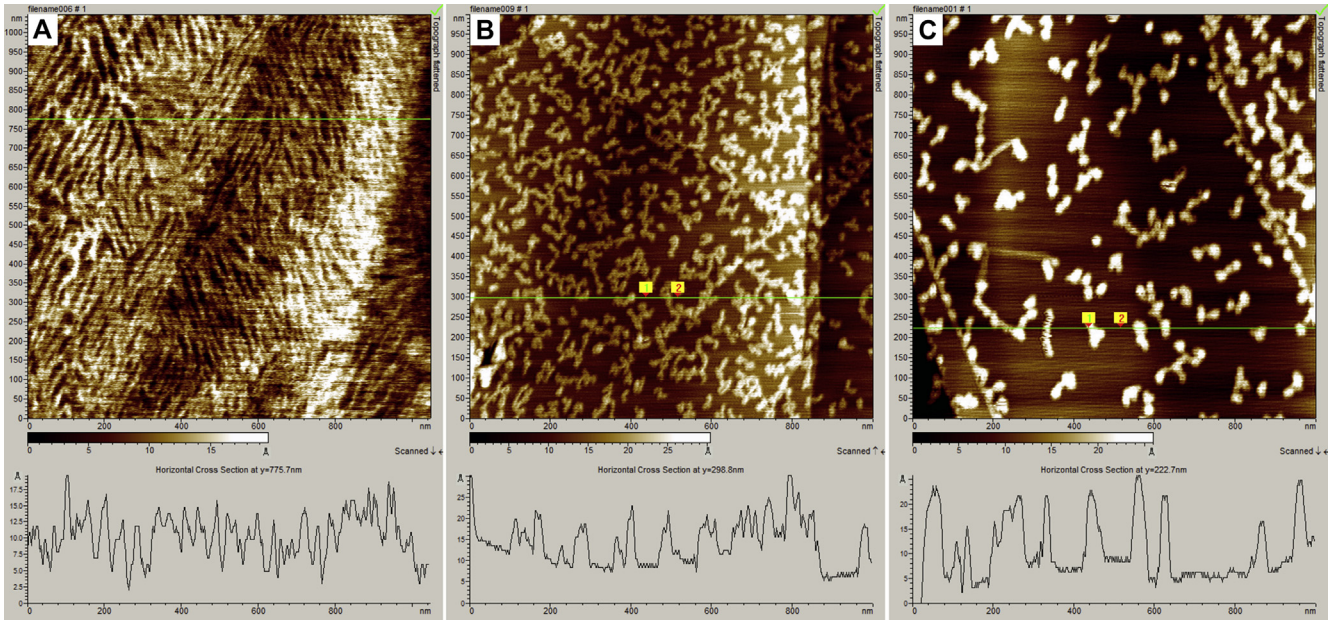

Fig. 3. The typical AFM topographic image of Oligodeoxynucleotide 5′-AGAGAGAGAG-3′ [$(AG)_5$] (2 ng/μl) and their assembly structures on HOPG surface after incubation with 83 μmol/l cisplatin for the predefined time. (a) Oligodeoxynucleotide only (b) incubation with cisplatin for 30 min (c) incubation with cisplatin for 1 h.

interconnected particles are believed to be related to formation of *cis*-[Pt(NH3)2{d(ApG-N7(1),N7(2))}] 1,2-intrastrand adducts. The interconnected particles in Fig. 3c have the height of 1.70 ± 0.06 nm and horizontal diameter around 30 nm. These dimensions suggest that each particle should be composed of at least 5 oligonucleotides. These observations suggest that activated cisplatin lead to assembly of the oligonucleotide particles through coordination and electrostatic interactions.

We propose that the structural changes of linear dsDNA induced by activated cisplatin follow the mechanism illustrated in Fig. 4. Activated cisplatin coordinates with the N7 of guanine or adenine on DNA to form monoadducts. Electrostatic repulsion between the neighboring DNA molecules is overcome by the presence of activated cisplatin, a divalent cation which can form a charge bridge between the negatively charged backbones of the different DNA. These interactions facilitate cross-linking of neighboring DNA molecules as indicated in Fig. 2a and illustrated in Fig. 4c. As the incubation time increases, monoadducts can further develop into intrastrand diadducts, which bend and wind local DNA strands at the binding site resulting in DNA kinks pictured in Fig. 4e. These DNA kinks correspond to the beads-on-string structures pictured in Fig. 2b. Finally, these structures assemble into large aggregates as shown in Fig. 4f.

To our knowledge, these uniform beads-on-string structures and assembly network have not been well-characterized previously. In our recent study, we obtained worm-like structures and compact particles in the DNA platination process but did not observe these interesting configurations at different concentrations of cisplatin [11]. A possible reason is that the cisplatin was not activated by $AgNO_3$ precipitate method in our previous study [11]. It is believed that the activation results in more efficient binding. More recently, micro-rod structures of long linear DNA induced by low-concentration cisplatin have been reported [9]. It is indicated that the micro-rod structures were formed mainly within

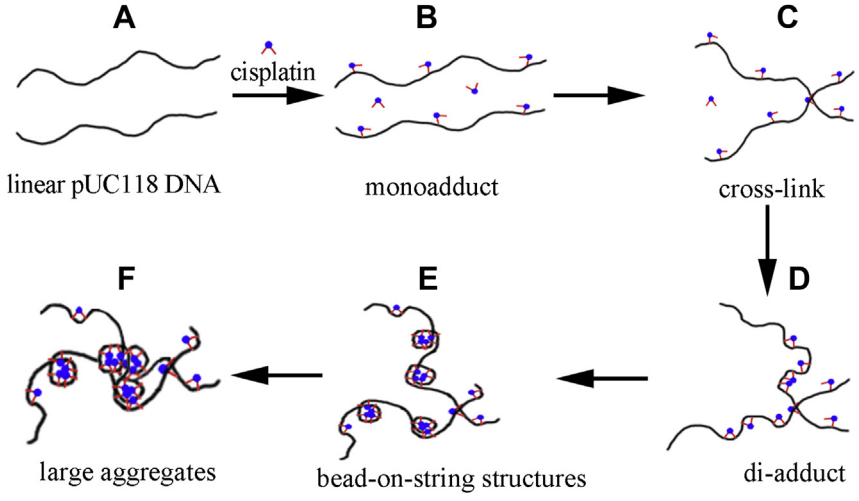

Fig. 4. Illustration of the platination process of long linear DNA induced by the activated cisplatin.

individual molecule. They did not observe DNA networks such as those seen in the present study, this is likely due to the use of relative low concentration of DNA and cisplatin [9]. Our present results clearly demonstrated that DNA–DNA interactions were quite strong at the relatively high concentration of DNA and cisplatin. DNA network structures remained even after formation of some large aggregates as shown in Fig. 2c. The arrangement of the DNA and cisplatin inside of bead-and-string structures is still unclear. Further research using other strategies such as molecular simulation may be helpful for understanding this novel structure. The present finding is very important because formation of these kinds of cisplatin-DNA structures may influence the activity and cytotoxicity of cisplatin.

Conflict of interest

None.

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Transparency document

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