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The Interaction of Oxaliplatin(Pt(oxalate)(1R, 2R-cyclohexanediamine)) with DNA Visualized by an Atomic Force Microscope

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The structural changes of DNA caused by $PtCl_2$(diaminocyclohexane (DACH)) were observed by an atomic force microscope (AFM). The AFM image of pUC19 DNA treated by *Eco RI* show that the DNA is shaped like a string. The AFM image of DNA treated with $PtCl_2$(DACH) at a ratio of platinum compound / base pair (r)=1.0 or 0.1 is very different from that of DNA and shows an ellipsoidal sphere in which the string of DNA was rolled up. The DNA treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.01 is also shaped into a ball, but the diameter of the ball is larger than that treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=1 or 0.1. It is known that, when the platinum complex binds to the double helix of DNA, a kink occurs in the DNA. The dramatic changes of the shape of the DNA from a string to a sphere were occurred by accumulating the DNA kink induced by the platinum complex.

Key words: anticancer platinum complex, AFM image, DNA, oxaliplatin

Of the many thousands of platinum compounds synthesized over the last 30 years, only a few dozen have reached preclinical or early clinical development. Of these, until recently, only cisplatin and carboplatin were available. Both have similar clinical properties. Additionally, the diaminocyclohexane(DACH) platinum family of compounds was shown in the early 1970s to have a different preclinical activity profile [1]. In the mid 1970s, Professor Kidani described the relationship between the stereo-isomeric specificity of DACH platinum compounds binding to DNA and cytotoxicity. And he also showed that oxaliplatin (Pt(oxalate)(DACH)) has high anticancer potential [2]. In the late 1980s, Professor Mathé had the foresight to bring oxaliplatin to the clinic [1]. Now oxaliplatin is widely using as anti-cancer drug throughout the world.

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Our understanding of the relationship between the "structure" and "function" of bio-molecules, has been very important [3]. It has been shown that the final target of the platinum complex is DNA. In life science, it has been of great interest to view the DNA structure bound by the platinum compound, an anticancer agent, on a nanometer-scale. The atomic force microscope is a very convenient tool for viewing bio-molecules. In this paper, we describe the structural changes of DNA caused by $PtCl_2$(DACH) based on observation with an atomic force microscope.

Material and Methods

The linear DNA was obtained by treating pUC19(2.69Kbp) with *Eco RI*. Pt(DACH)Cl_2 was used as a platinum complex and dissolved in water. The platinum content was measured by flame less atomic absorption spectrophotometry. The concentrations of DNA base were determined by a UV spectrophotometer using the molar absorptivity. The linear DNA dissolved in PBS buffer was mixed with Pt Cl_2 (DACH) at the ratio of platinum compound / base pair (r)=1, 0.1, 0.01, and 0.001. The mixture solution of DNA and platinum compound was incubated for 1 hr at 37 °C. The unbound platinum compound remaining in solution was removed by dialysis against distilled water. The adduct of DNA-Pt(DACH) was diluted by distilled water and 10 micro-liters of the solution containing DNA-Pt(DACH) was placed onto a mica and then dried at room temperature for 1 day. The sample was imaged in a SPI3700 AFM (Seiko Co. Ltd.) operating in tapping mode I air at a scan rate of 1-3 Hz. The DFM probes were 125 mm-long monocrystalline silicon cantilevers with integrated conical-shaped Si tips with an average resonance frequency f_0=312 KHz and spring constant C=31 N/m.

Results and Discussion

A typical large-scale AFM image of pUC19 DNA treated by EcoR I is shown in Fig. 1A. A high-resolution image of Fig. 1A is also shown in Fig. 1B. These figures clearly show that the DNA is shaped like a string.

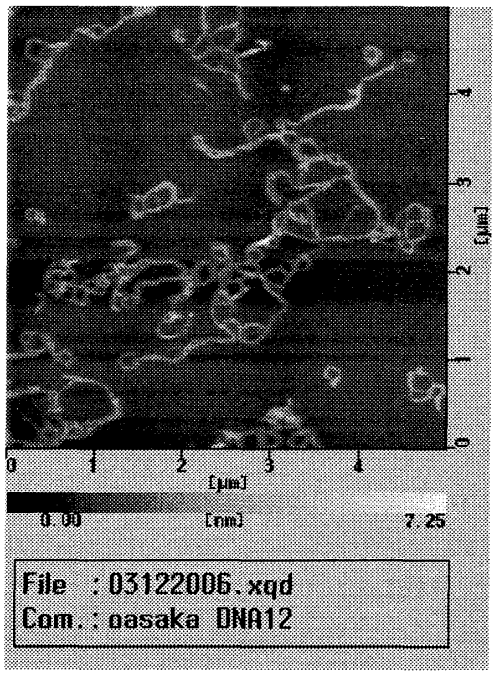


Fig. 1A DNA of pUC19 treated with Eco. RI

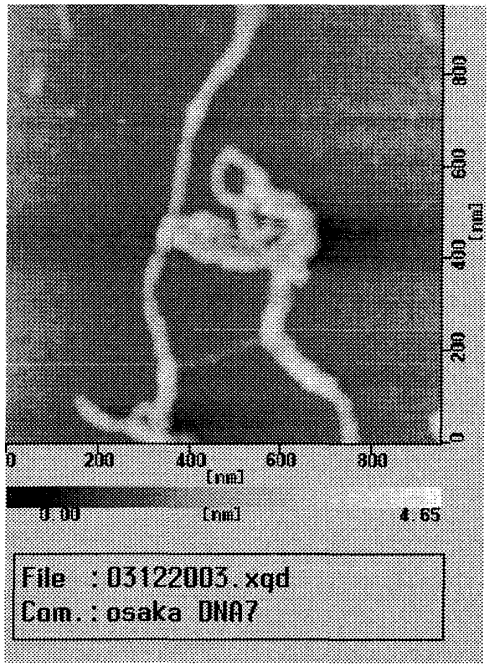


Fig. 1B The high-resolution image of pUC19 treated with Eco. RI

The AFM images of DNA treated with $PtCl_2$(DACH)

In Fig. 1B, the width of the string of DNA is almost 20 nm. The value is larger than that (2~3 nm) expected from DNA. The AFM method was used in this experiment so that a wider width of DNA string could be imaged in Fig. 1B.

The AFM image of DNA treated with $PtCl_2$(DACH) at a ratio of platinum compound / base pair (r)=1.0 is shown in Fig. 2A. In Fig. 2A, the shape is very different from that of DNA. A high-resolution image of Fig. 2A is shown in Fig 2B. The AFM image of DNA treated with $PtCl_2$(DACH) shows an ellipsoidal sphere in which the string of DNA was rolled up. The nine ellipsoidal spheres of the DNA are shown in Fig 2A, and the shapes are very similar to each other. These findings indicate at each elliptical sphere was rolled from each pUC19 DNA string. In Fig. 2B, the high-resolution image of the ellipsoidal sphere shows that the surface is even. This image indicates that the string of the DNA was irregularly rolled up.

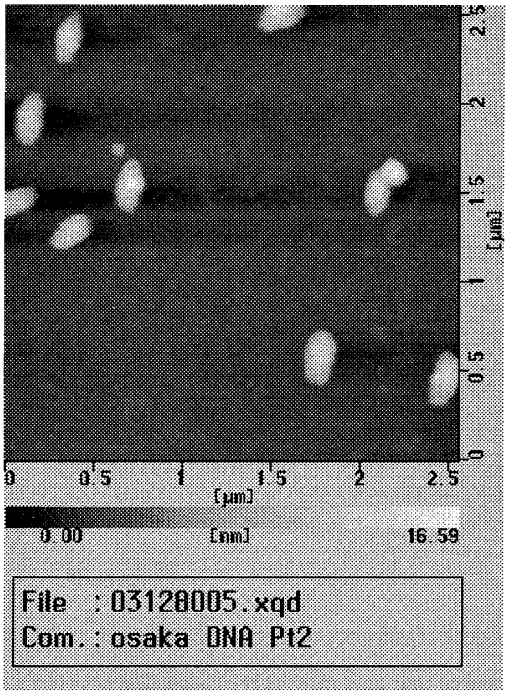


Fig. 2A. The AFM image of DNA(pUC19) treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=1.0

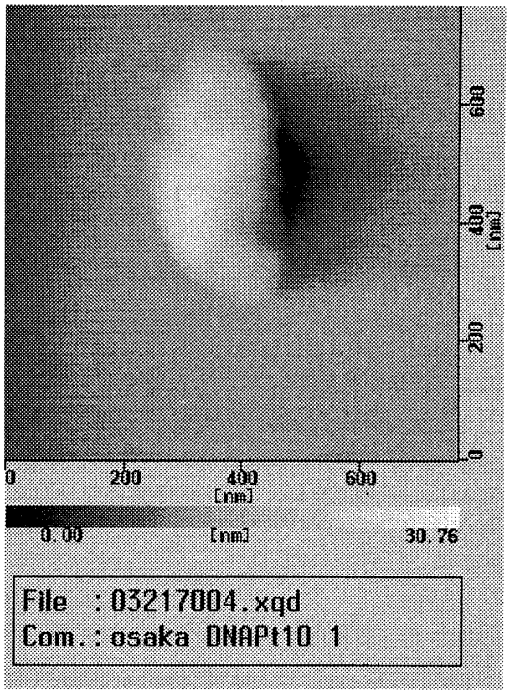


Fig. 2B. The high-resolution image of DNA(pUC19) treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=1.0

The AFM image of the DNA treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.1 was also collected, and the shape of the DNA treated with $PtCl_2$(DACH) at r=0.1 was very similar to those shown in Fig. 2A or 2B.

The AFM image of DNA treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.01 is shown in Fig. 3. In Fig. 3, the shape is very different from that of the DNA treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=1 or 0.1 (Fig. 1 or Fig. 2). In Fig. 3, the DNA treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.01 is shaped into a ball, and the diameter of the ball is larger than that treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=1 or 0.1. The diameter of the ball in Fig. 3 is approximately 0.5 μm, while the lengths of the ellipsoidal sphere of DNA treated with $PtCl_2$(DACH) at the ratio of

platinum compound / base pair (r)=0.1 is approximately 0.2 x 0.4 μm in Fig. 2 B. The large ball in Fig 3 may indicate that the string of DNA was loosely rolled at the ratio of platinum compound / base pair (r)=0.01, while the small ellipsoidal sphere in Fig. 2A or B may indicate that the string of DNA was tightly rolled.

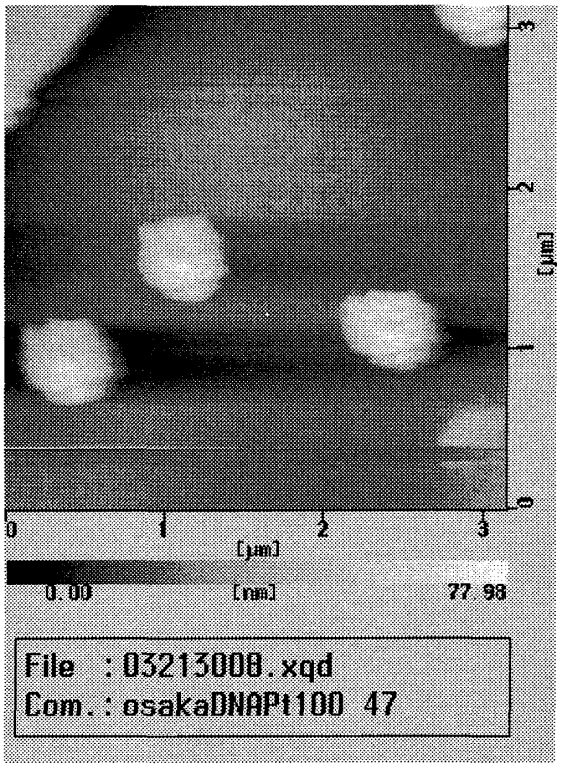


Fig. 3 The AFM image of DNA(pUC19) treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.01.

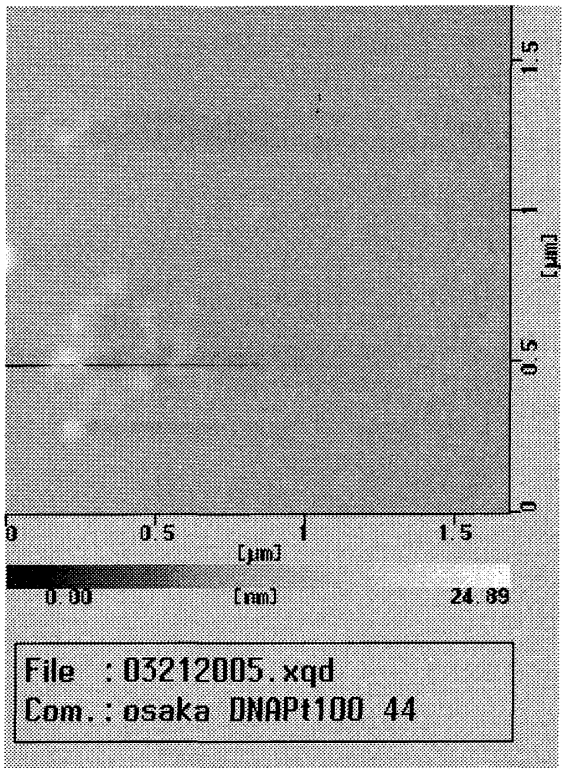
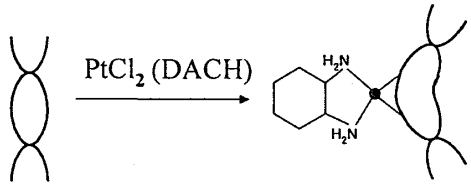


Fig. 4 The AFM image of DNA(pUC19) treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.001.

The AFM image of DNA treated with $PtCl_2$(DACH) at the ratio of platinum compound / base pair (r)=0.001 is shown in Fig. 4. The DNA image in Fig. 4 is not as clear, but it indicates that part of the DNA was like a string and the other part like a sphere in which the DNA was rolled up.

The AFM images of the DNA in Figs. 1~4 show that the string of the DNA was tightly rolled in response to the increase in the contents of the platinum-DNA adduct. Why are these dramatic changes of the shapes of DNA induced by increases in the content of the platinum-DNA adduct ? Lippard has showed that when the platinum complex binds to the double helix of DNA, a kink occurs in the DNA, as shown in Scheme 1 [4]. When many platinum complexes bind to DNA, many of these DNA kinks are caused. Therefore, the shape of the DNA dramatically changes from



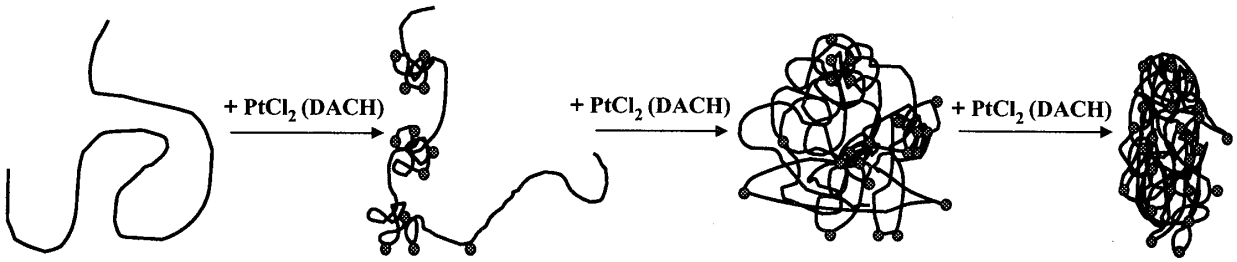
Double helix of DNA

The kink of DNA occurred by Pt(DACH)

Scheme 1. The kink of DNA induced by binding of the platinum complex.

The AFM images of DNA treated with $PtCl_2$(DACH)

a string to a sphere. The gradual change in shape of the DNA induced by the platinum-DNA adduct are shown as Scheme 2.



Scheme 2. The gradual conformational changes with increases in the content of the platinum adduct in DNA. The platinum complex is shown by a gray circle, and DNA is shown by a curved line.

Acknowledment

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