Direct measurement of interaction forces between a platinum dichloride complex and DNA molecules

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Abstract The interaction forces between a platinum dichloride complex and DNA molecules have been studied using atomic force microscopy (AFM). The platinum dichloride complex, di-dimethylsulfoxide-dichloroplatinum (II) ($\text{Pt}(\text{DMSO})_2\text{Cl}_2$), was immobilized on an AFM probe by coordinating the platinum to two amino groups to form a complex similar to $\text{Pt}(\text{en})\text{Cl}_2$, which is structurally similar to cisplatin. The retraction forces were measured between the platinum complex and DNA molecules immobilized on mica plates using force curve measurements. The histogram of the retraction force for λ -DNA showed several peaks; the unit retraction force was estimated to be 130 pN for a pulling rate of 60 nm/s. The retraction forces were also measured separately for four single-base DNA oligomers (adenine, guanine, thymine, and cytosine). Retraction forces were frequently observed in the force curves for the DNA oligomers of guanine and adenine. For the guanine DNA oligomer, the most frequent retraction force was slightly lower than but very similar to the retraction force for λ -DNA. A higher retraction force was obtained for the adenine DNA oligomer than for the guanine oligomer. This result is consistent with a higher retraction activation energy of adenine with the Pt complex being than that of guanine because the kinetic rate constant for retraction correlates to $\exp(F\Delta x - \Delta E)$ where ΔE is an activation energy, F is an applied force, and Δx is a displacement of distance.

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1 Introduction

Cisplatin (*cis*-diamminedichloroplatinum (II), $(\text{Pt}(\text{NH}_3)_2\text{Cl}_2)$) was the first platinum complex developed as an antitumor agent; it is still widely used [1, 2]. Cisplatin binds to DNA molecules and blocks the transcription of nucleotide sequences. The binding sites of cisplatin are the N7 atoms of guanine or adenine in DNA molecules. The structures of DNA-cisplatin adducts have been studied by NMR spectroscopy and X-ray crystallography. It has been reported that the major adducts are intrastrand cross-links at the d(GpG) and d(ApG) sites [3, 4].

The changes in DNA conformation upon binding with platinum antitumor drugs, such as cisplatin and other Pt(II) and Pd(II) complexes, have been directly characterized by atomic force microscopy (AFM); these DNA conformation changes have been studied under various conditions [5–13]. AFM imaging results have directly revealed the conformational changes induced in DNA by cisplatin, supporting the results obtained by other nondirect analytical methods.

AFM has been utilized for affinity evaluation *in vitro*. This can be accomplished by two methods. One is a single molecular pulling method, where polymer molecules bound to a plate are crosslinked with inside molecules, and the retraction force is measured by pulling on a polymer molecule that is bound to the tip of a cantilever. The other method involves detecting an adhesive force between a molecule immobilized on a plate and a molecule immobilized on the tip of a cantilever by measuring a force curve. Many researchers have examined these force curves and have succeeded in evaluating the interactions between a biomolecule immobilized on a cantilever tip and another biomolecule on a plate, such as DNA–DNA, protein–protein, and biotin–avidin interactions [14–26].

To study the interaction forces of cisplatin, the single molecular extension method has been applied using AFM [27, 28] and magnetic tweezers [29, 30]. In these studies, the differences in the force-distance curves were analyzed by stretching DNA molecules that were treated and not treated with cisplatin. Although the B-S transition of DNA (structure change from B-DNA to S-DNA) should be separate from the force action, and the binding sites of cisplatin are very close in DNA molecules; a force step of 73 pN was reported for pulling rates of 0.1 to 5 $\mu\text{m/s}$ with cisplatin in the force curves [27]. Cross-evaluation by a different method is important; however, to our knowledge, there are no reports of immobilizing cisplatin to an AFM probe and measuring the retraction forces between cisplatin and DNA.

The platinum complex $\text{Pt}(\text{en})\text{Cl}_2$ has a *cis* steric structure coordinating two amino groups; it can bind DNA molecules similarly to cisplatin. The properties of $\text{Pt}(\text{en})\text{Cl}_2$ have been studied by analyzing its adduct products with DNA [31, 32].

In this paper, we have immobilized a dichloroplatinum complex that is similar in structure to $\text{Pt}(\text{en})\text{Cl}_2$ on an AFM probe and measured the retraction forces between the probe and DNA immobilized on a mica plate. The interaction forces were evaluated, and the dependence of the forces on the DNA bases was studied from histograms of the obtained retraction forces.

2 Experimental section

2.1 Reagents

Cisplatin, λ -DNA (48.5 kbp, 0.5 $\mu\text{g}/\mu\text{l}$), hydrochloric acid (1 mol/l), and acetonitrile were obtained from Wako Pure Chemical Industries Ltd. 3-aminopropyltriethoxysilane (APTES) was obtained from Tokyo Chemical Industry Co. Silver nitrate, potassium tetrachloroplatinate (II) ($\text{K}_2[\text{PtCl}_4]$), and ethylenediamine were obtained from Sigma-Aldrich. Dithiobis(succinimidyl undecanoate) (DSU) was obtained from Dojindo Molecular Technologies. Dimethyl sulfoxide (DMSO) was obtained from Merck. DNA oligomers (59 b) were obtained from Tsukuba Oligo Service. All reagents were used without further purification, and all reactions were performed at room temperature unless otherwise noted.

2.1.1 Preparation of di-dimethylsulfoxide-dichloroplatinum (II) ($\text{Pt}(\text{DMSO})_2\text{Cl}_2$)

Five hundred microliter DMSO and 100 mg potassium tetrachloroplatinate (II) ($\text{K}_2[\text{PtCl}_4]$) were mixed in a lightproof bottle; 82 mg silver nitride was added, and the mixture was mixed with a stir bar for 48 h. The reaction in the solution proceeded as follows:

Figure 1a shows this reaction and the chemical structures of $\text{Pt}(\text{DMSO})_2\text{Cl}_2$. After the reaction, the supernatant in the bottle containing $\text{Pt}(\text{DMSO})_2\text{Cl}_2$ was separated into another lightproof bottle. The $\text{Pt}(\text{DMSO})_2\text{Cl}_2$ solution was mixed with water in a 1:1 ratio, and this mixture was used for the next experiment.

a

b

Fig. 1 Procedure for (a) preparing $\text{Pt}(\text{DMSO})_2\text{Cl}_2$ and (b) immobilizing the platinum complex on the cantilever tip. The Au surface was subsequently treated with (i) dithiobis(succinimidyl undecanoate) (DSU), (ii) ethylenediamine (en), and (iii) $\text{Pt}(\text{DMSO})_2\text{Cl}_2$

2.2 Immobilization of the platinum complex on the cantilever probe

The platinum complex was immobilized on the tip of a cantilever using DSU and ethylenediamine. Figure 1b shows the immobilization steps. The silicon nitride cantilever (TR800PB-1, Olympus Corp.) covered with Au was immersed in 1 mol/l HCl solution for 10 min and then rinsed with acetonitrile. The cantilever was treated with DSU in acetonitrile (1 mmol/l) and was maintained in the solution for 2 h to cover the surface with a self-assembled monolayer (SAM) (Step (i) in Fig. 1b). After rinsing with acetonitrile, the cantilever was treated with 1 mmol/l of ethylenediamine dissolved in acetonitrile for 1 h and was then rinsed with acetonitrile and water (Step (ii) in Fig. 1b). Next, the cantilever was treated in the $\text{Pt}(\text{DMSO})_2\text{Cl}_2$ solution for 2 h and rinsed with water (Step (iii) in Fig. 1b). After the immobilization procedure, the cantilever was reacted with Tris-HCl buffer (10 mmol/l, pH 8) for 12 h to terminate the remaining succinimidyl groups on the SAM. The cantilever was rinsed with water and maintained in a desiccator. Ultimately, the platinum dichloride coordinated to two amino groups, similar to cisplatin.

2.3 X-ray photoelectron spectroscopy (XPS)

XPS was performed on a JPS-9200 (JEOL) instrument to confirm the immobilization reaction of the platinum complex. The spectra were acquired using unmonochromatized $\text{MgK}\alpha$ radiation (photon energy = 1253.6 eV) at a pass energy of 50 eV and were averaged for 20 scans. The peak positions were assigned by referencing the C1s peak to a binding energy of 285 eV.

2.4 Immobilization of DNA on mica plates

A mica plate ($5 \times 5 \text{ mm}^2$) was bound to a glass plate with epoxy resin. The mica plate surface was freshly cleaved with Scotch tape, and the plate was immersed in a 0.005% solution of APTES in water for 15 min. After rinsing with water, the mica plate was dried in a vacuum chamber for 15 min and then heated to 120 °C for 5 min in the chamber. After the mica plate was cooled to room temperature, 0.05 $\mu\text{g}/\mu\text{l}$ DNA solution was placed on the mica surface for 2 min (in the case to image the cisplatin treated λ -DNA, DNA solution was placed first, and then cisplatin solution was added and kept for 2 min). The DNA solution was dropped from the mica surface; the plate was rinsed gently with H_2O and then treated in a vacuum chamber for 15 min and at 120 °C for 5 min.

2.5 AFM imaging and force curve measurements

AFM imaging and force curve measurements were performed using an SPI4000 AFM (Seiko Instruments). DNA imaging was performed in water. For the imaging in water, a silicon nitride cantilever probe (TR800PB-1, Olympus Corp.) was used in contact mode. The mica plate with immobilized DNA was placed in a fluid well containing phosphate buffer (10 mmol/l, pH 7.4). To treat the DNA with cisplatin, 10 mg of cisplatin was dissolved in 20 ml of normal saline. The cisplatin solution was used immediately without storing.

The force curve measurements were performed using the AFM probe modified with the platinum complex in a fluid well. The spring constant of the AFM cantilever was calibrated by the thermal vibration method [33], and the force curve was recorded at a velocity of 60 nm/s.

The movement of the cantilever was suspended for 1 s at the reverse point, which is the closest point between the tip and the plate. Each force curve was measured at different points on the plate, and each point was manually set on the sample plate in a square (ca. 800 nm in width). A few hundred force curves were recorded at different positions on the sample plate (500 times for the APTES treated mica, 1000 times for λ -DNA immobilized mica, and 150 times each for the four different DNA oligomers), and the retraction forces were aggregated from each force curve. The histograms of the measured retraction forces are presented as probabilities (%), which are the number of counts divided by the total number of counts.

3 Results and discussion

3.1 Confirmation of platinum complex immobilization

Figure 2 shows the XPS spectra of the Au-coated cantilever and the cantilever with the platinum complex immobilized on its Au surface. For the Au-coated cantilever, the main peaks of Au, at 84 and 88 eV, were observed. For the platinum complex-immobilized

Fig. 2 XPS spectra for (a) the Au-coated cantilever and (b) the cantilever with Pt(en)Cl₂ immobilized on the Au surface

cantilever, additional peaks for Pt were observed. These peaks correspond to the main peaks of Pt at 71 and 74 eV; they were shifted 2 eV higher due to coordination with higher electronegativity atoms. This result indicated that the platinum complex was successfully immobilized on the cantilever.

3.2 AFM imaging of DNA

To confirm the immobilization of DNA on the mica plate, AFM imaging was performed. Figure 3a shows the AFM image of λ -DNA on a mica plate without cisplatin treatment. DNA shows a mainly linear structure on the sample plate. This result is contrary to a report that described a bending DNA structure on mica under equilibrium conditions by adsorption kinetics [34]. However, we have shown similar linear DNA images in previous reports [35, 36]. The formation of a linear structure can be explained by the fact that λ -DNA has short single-stranded regions at its ends and these regions are more attractive to a mica surface than double-stranded regions; thus, a liquid flow during the immobilizing and rinsing processes results a linear structure.

For the cisplatin-treated λ -DNA, a structural change in the DNA was observed, as shown in Fig. 3b. The DNA acquired a circular shape upon treatment with cisplatin. This demonstrates that cisplatin bound to and bent the DNA. In the experiments, we placed the DNA solution on the mica surface first and then added cisplatin solution where the DNA adsorption to the mica surface and cisplatin binding process occurred simultaneously. In contrast, when a mixture of DNA and cisplatin was immobilized, the DNA formed an aggregated structure as small particles. Thus, we mixed the two solutions on the mica surface.

For the force curve measurements, λ -DNA was immobilized at a concentration ten times higher than the concentration normally used for imaging DNA to increase the probability of binding between DNA and the platinum complexes. Figure 3c shows the immobilized λ -DNA image used for the force curve measurement; the image was acquired on the mica plate in solution. In Fig. 3c, it can be seen that the DNA molecules are immobilized due to the thick density of the DNA. The tip of the probe is expected to interact with DNA molecules during every step of the force curve measurements.

Fig. 3 AFM images of λ -DNA on a mica plate without cisplatin treatment (a), with cisplatin treatment (b), and with a ten times greater DNA concentration for force curve measurements (c)

3.3 Force curve measurements of λ -DNA

During force curve measurement, the tip of the cantilever approaches the sample surface, and after contacting the surface, the tip retracts from the surface. When the tip contacts the sample surface, several specific or nonspecific bonds form between the tip and surface molecules where deformation or movement may occur on the sample surface. During the retraction process, the cantilever bends downward because the tip is trapped by these bonds. When the bonds are broken because of gradual decrease in the contacting area or deformation, the cantilever bend is removed. The retraction force is obtained from the spring constant of the cantilever and the change in the cantilever bending displacement when the bonds break.

Figure 4 shows the typical force curves for APTES-treated mica surfaces without DNA (Fig. 4a) and with λ -DNA (Fig. 4b) measured with the platinum complex-immobilized probe in PBS. For the mica surface without DNA, a single retraction force was observed in the force curve (Fig. 4a). In contrast, multiple peaks were observed for the λ -DNA immobilized surface (Fig. 4b). For the origin of the peaks, if the probe and DNA remain bound and the DNA detaches from the mica surface, the force curve must be a long-range response over several micrometers because we used λ -DNA, which is 48.5 kbp (16 μm long), in this study. If a few nanometers separations occurred between the mica and DNA, they occurred before the separation between DNA and the platinum complex because λ -DNA does not completely separate from the surface during the force curve measurement. Therefore, we concluded that the force peaks appearing in Fig. 4b are due to the retraction between the probe and DNA.

The single peak of the retraction force that is observed for the mica surface without DNA (Fig. 4a) should be caused by nonspecific bonds. As this peak is larger than that for a single nonspecific bond, the retraction force is formed from the sums of the forces of multiple nonspecific bonds. Similarly, the first peak of the retraction force observed in Fig. 4b is likely caused by nonspecific bonds including those between DNA and the probe and between DNA and the mica surface. The purpose of this study is to detect the single-molecular-level force. The first large peak is too large for a single-molecular-level force. Therefore, the first peak of the force curve was omitted for the force counting in all force curve measurements. For the force peak value measurement, the retraction forces α and β , shown in Fig. 4b, were recorded as values of the retraction force.

Figure 5 shows the histograms of the retraction forces obtained for the APTES treated-plain mica surface (Fig. 5a) and the λ -DNA-immobilized mica surface (Fig. 5b). In Fig. 5, the

Fig. 4 Typical force curves for (a) the mica surface without DNA and (b) the mica surface with immobilized λ -DNA

Fig. 5 Histograms of the retraction forces obtained from (a) APTES-treated mica and (b) λ -DNA-immobilized mica

histograms were plotted for each 10 pN range. For the APTES-treated mica surface (Fig. 5a), the peak in the retraction force histogram was 60 pN. This value corresponds to previously reported values for nonspecific interaction forces [24]. In Fig. 5a, a very small peak can be seen at approximately 120 pN. This is produced by multiple nonspecific bonds that are retracted simultaneously. In Fig. 5a and b, a peak at 60 pN was observed; also, a peak at 130 pN was observed in Fig. 5b. The peak at 130 pN was expected due to the retraction between DNA and the platinum complex because the obtained unit force 130 pN is approximately two times greater than the reported value of 73 pN for the DNA pulling method by Krautbauer et al. [27]. This is also supported by the chemical structure of the probe shown in Fig. 1b. The probe has a similar structure to cisplatin, and many reports have demonstrated that cisplatin binds to two sites on DNA.

There is a slight difference between the force 130 pN and twice the value of Krautbauer's result 146 pN. This may be caused by the measuring condition. Some reports have explained the difference caused by the pulling rate in that a higher pulling rate results in a greater retraction force [37, 38]. The pulling rate in the referenced study is faster than in our experiments; thus, the retraction force in our experiments can be lower than Krautbauer result [27]. This supports that the force obtained in this work is the sum of the forces of the two binding sites of the platinum complex.

Additionally, multiple peaks were observed at 260, 390, and 520 pN. These can be attributed to the simultaneous retraction of multiple additional binding molecules. This result indicates that 130 pN is the unit attractive force for DNA and the platinum complex.

3.4 Retraction force measurements for DNA oligomers

The force curves were measured for four homogeneous DNA oligomers of adenine, thymine, guanine, and cytosine, immobilized on mica plates. The force curves for the immobilized samples of the DNA oligomers of guanine and adenine frequently showed retraction forces; however, the force curves for thymine and cytosine showed very few retraction force signals apart from the nonspecific retraction force of 60 pN.

Figure 6 shows the histograms of the retraction forces for DNA oligomers of the four bases. As the density of the immobilized DNA was half lower than that for λ -DNA, the probability of the appearance of a retraction force was slightly lower than that for λ -DNA. In the histograms

Fig. 6 Histograms of the retraction forces for homogeneous DNA oligomers of (a) adenine, (b) thymine, (c) guanine, and (d) cytosine

for adenine and guanine, the peaks at approximately 60 and 120 pN were observed. In contrast, the 60 pN peaks were mainly observed for the thymine and cytosine DNA oligomer samples (Fig. 6a and c), and almost no signals were observed at 120 pN as well as in the histogram without DNA (Fig. 5a) (the arrows at 120 pN in Fig. 6a and c do not indicate peaks, but the position of the force values). These results support that the platinum complex binds selectively to guanine and adenine. In the histogram of guanine, the peak position of 120 pN was similar to, but slightly smaller than, the value obtained from the histogram of λ -DNA. This can be explained by the fact that the DNA oligomer consisted of single-stranded DNA; thus, the binding energy between the platinum complex and DNA is expected to shift.

Comparing the histograms of guanine and adenine, the peak at approximately 120 pN for the adenine sample spreads to a slightly higher range than that for guanine. This can be explained by the difference in the activation energies of the adducts formed between the platinum complexes and adenine and guanine; this is because the activation energy for retracting the Pt complex from adenine is higher than that for retracting the Pt complex from guanine [39]. A higher activation energy results in a higher retraction force and lower binding and rebinding probabilities because the kinetic rate constant for retraction correlates to $\exp(F\Delta x - \Delta E)$ where ΔE is an activation energy, and F is an applied force. Thus, the retraction force correlates to the activation energy under the expectation that the displacement of distance (Δx) between the probe molecule and DNA are similar [37].

4 Conclusions

A platinum complex was successfully immobilized on an AFM cantilever in the form of Pt(en)Cl₂. The retraction force between the platinum complex and λ -DNA molecules was estimated as 130 pN, which is the sum of the retraction forces of the two binding sites. This force value is similar to previous reports. For single DNA base oligomers, the force curves for the guanine and adenine DNA oligomers frequently showed retraction forces. For adenine, a higher retraction force was observed; this is due to the difference in the activation energies of the platinum complex and the different DNA bases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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