A study of the effects of cisplatin on nucleosomal DNA using magnetic tweezers

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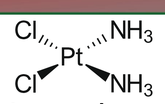
Abstract

The hallmark of cancerous cells is their incessant cell division, and they thus push and invade intact normal cells. Chemotherapeutic agents help to cure cancers by inducing apoptosis in the affected cells. Cisplatin, one of the most effective cytotoxic agents and displaying clinical activity against various tumors, has been widely used in cancer chemotherapy for decades. The well-known mechanism of cisplatin is that the drug binds and kinks DNA via crosslinking, which can lead to cell death. However, the working mechanism of cisplatin on the physiological condition has not been fully understood yet. In reality, cellular anionic species suppress cisplatin activity by forming non-reactive complexes with cisplatin. Besides, recent studies found several intriguing roles of histones in cisplatin's anti-cancer effect. Here, we reconstituted nucleosomes on a single DNA tether molecule under a physiological ionic condition by using a histone chaperone called NAP1. We then studied the effects of cisplatin on the reconstituted nucleosomal tether molecule under various ionic conditions with magnetic tweezers. Surprisingly, the reduced activity of cisplatin under physiological ionic conditions is still sufficient to eliminate the conformational flexibility and reversibility of nucleosomal DNA by fixing its condensed structure. The cisplatin-induced fastening of a nucleosomal DNA can therefore interfere with normal DNA metabolism. Our direct physical measurements shed new light on the understanding the mechanism of platinum-based anti-cancer drugs.

Introduction

◎ Cisplatin 

- Cisplatin is a chemotherapy drug widely used for various human cancers.
- It binds to DNA, causing local kink and unwinding.
- It interferes with cell division and kills rapidly growing tumor cells.
- Its activity may be blocked by anionic species present inside the cell.
- Recent studies have suggested that cisplatin targets chromatin.

◎ Motivation
- How can cisplatin be effective as an anti-cancer drug in a physiological ionic condition?

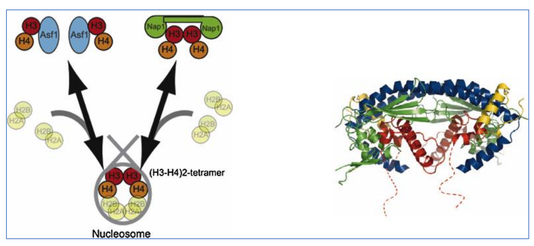
Methods

◎ Gel assay
- Reaction of DNA with 1 mM cisplatin at various ionic conditions
- Cisplatin titration under physiological salt condition

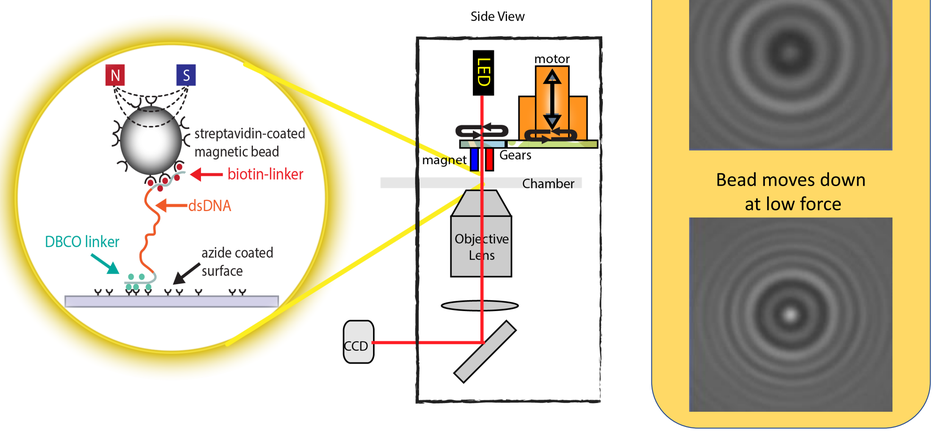
◎ Sample chamber preparation
- APTES coating after plasma treatment of slide glass
- MS(PEG)$_4$ used to passivate the chamber surface.
- Azide-PEG$_4$-NHS Ester and DBCO-dUTP are used to bind DNA(DBCO) to the (azide) surface.

◎ Nucleosomal DNA is constructed with histones and histone chaperone, NAP1.
- NAP1 binds a H3-H4 tetramer and two H2A-H2B dimers and sequentially loads them onto DNA.

Nucleosome Assembly Protein 1 (NAP1)

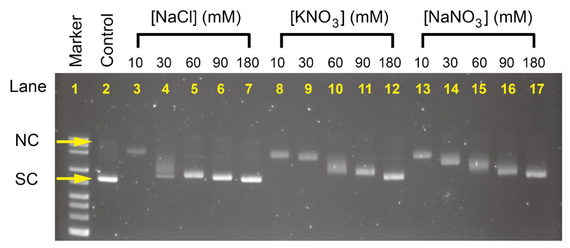


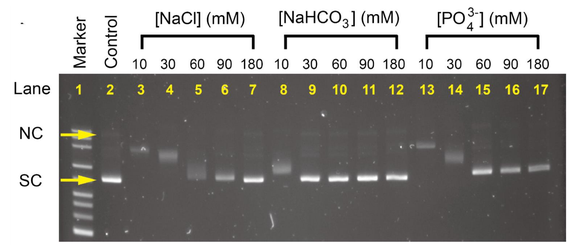
◎ Magnetic Tweezers(MT) can manipulate single biomolecules mechanically.
- MT can exert several pN of force to biomolecules.
- MT can twist DNA by turning the magnets.



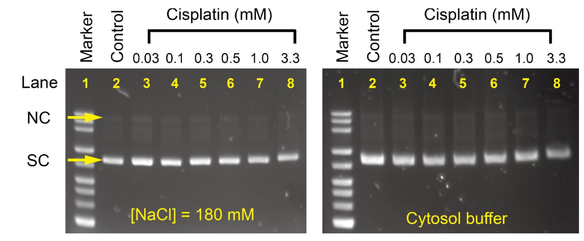
Results

◎ The degree of platination is low under high salt conditions.
- Cisplatin binds to DNA only at low ionic conditions.

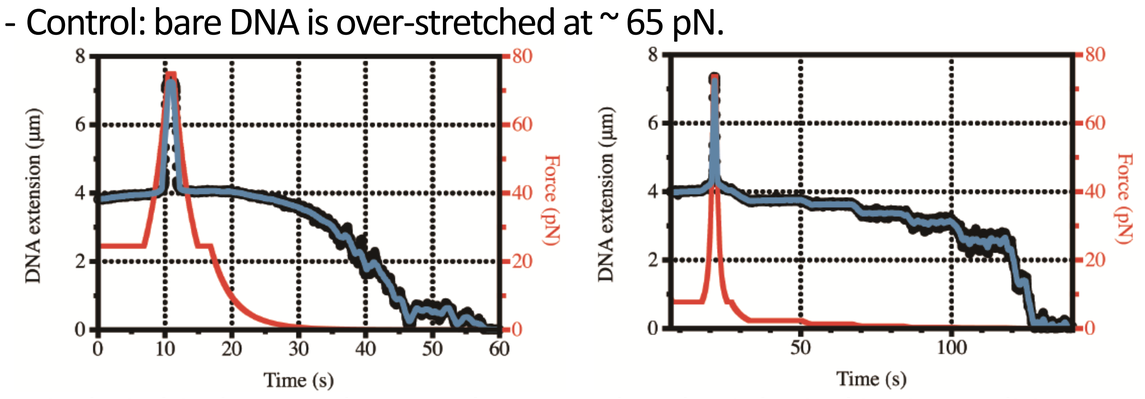




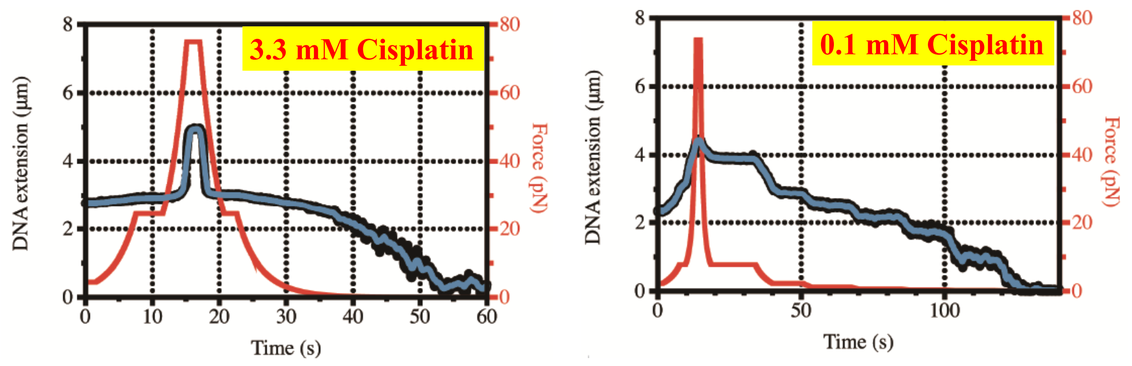
◎ In physiological ionic conditions, cisplatin binds weakly to DNA



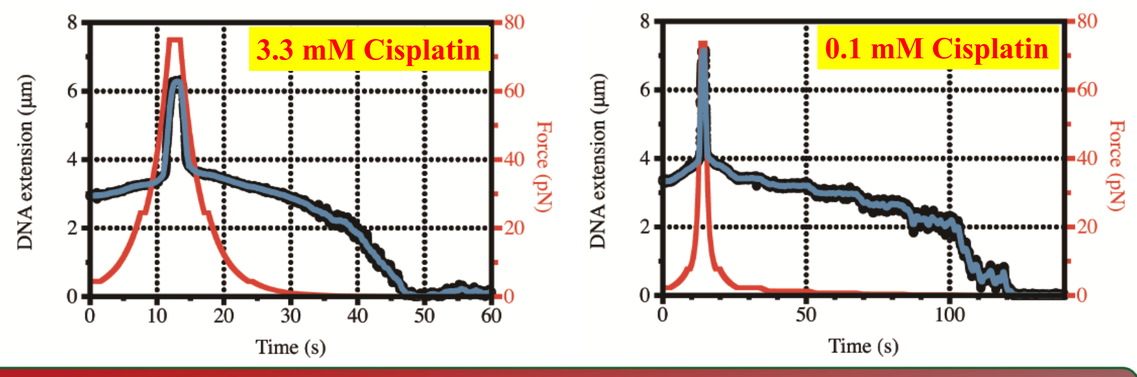
◎ Cisplatin alters nucleosomal DNA strikingly under physiological ionic conditions.
- Control: bare DNA is over-stretched at ~ 65 pN.



- Cisplatin binds to nucleosomal DNA under physiological ionic conditions
- Nucleosomal DNA is over-stretched but to less extent because of bound cisplatin



- Even under high salt conditions (3 M NaCl), some histones (likely crosslinked by cisplatin) remain bound to DNA ➔ Gene expression can be interfered.



Summary & Future works

◎ Cisplatin binds weakly to DNA under physiological ionic conditions but alters nucleosomal DNA effectively.

◎ We directly demonstrated that a clinical dose of cisplatin (0.1 mM) can fasten nucleosomal DNA at physiological ionic conditions.

◎ We plan to investigate the effects of other anticancer drugs on nucleosomal DNA in future.

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