Noncovalent Interactions

DOI: 10.1002/ange.201407093

Sequence and Chiral Selectivity of Drug–DNA Interactions Revealed by Force Spectroscopy**

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Abstract: *Differential binding force has been used to precisely characterize the mechanical effect of a drug molecule binding to a DNA duplex. The high-resolution binding forces measured by the force-induced remnant magnetization spectroscopy (FIRMS) enable the binding behavior of drug molecules with different chirality and DNA of various sequences to be distinguished. The sequence specificity of Hg^{2+} and daunomycin was revealed by force spectroscopy for the first time, and the results are consistent with those obtained by other techniques. Furthermore, the two isomers of* D,L*-tetrahydropalmatine showed selectivity for two different DNA sequences. One particular useful feature of this approach is that the small molecules under study do not require any labels.*

Interactions between small molecules, such as ions and drug molecules, and nucleic acids are widely encountered in biological functions and drug development.[1,2] Among the most important aspects in characterizing these systems are the sequence selectivity of the DNA and the conformational selectivity of the drug molecules. Various techniques have been extensively applied to study drug–DNA systems, including NMR spectroscopy,[3,4] second harmonic generation,[5] fluorescence spectroscopy,[6,7] circular dichroism,[8] UV melting,[9] X-ray diffraction,[10] magnetic tweezers,[11] atomic force microscopy (AFM),[12,13] and optical tweezers.[14–16] It remains challenging, however, to directly measure the binding strength of such interactions with sufficiently high force resolution, so that different drug–DNA interactions can be distinguished. Thus, few studies regarding DNA sequence selectivity and drug-molecule selectivity have been reported.

Recently, we reported the force-induced remnant magnetization spectroscopy (FIRMS) technique, which relies on external mechanical forces to resolve different noncovalent bonds on the basis of their binding strengths.[17,18] The force resolution has reached 1.8 pN, sufficient to resolve DNA duplexes with one base pair difference.[18] The external force may be a shaking force, a centrifugal force, or most recently an acoustic radiation force.[19] The FIRMS technique is based on the phenomenon that dissociated magnetic particles that are used for labeling biological molecules will have no net magnetic signal because of the randomization of their magnetic dipoles. Therefore, each bond dissociation is measured as a decrease in the overall magnetic signal. As FIRMS efficiently measures about 10^4 bonds at a time, but can only provide the initial and final states, it complements other force techniques such as AFM and optical tweezers for the study of the mechanochemistry of biological systems.

Here, we show a concept of using the differential binding force to precisely characterize the mechanical effect of a drug molecule binding to a DNA duplex. The high-resolution binding forces measured by the FIRMS technique enable the binding behavior of drug molecules with different chirality and DNA of various sequences to be distinguished.

Figure 1a shows a schematic representation of the method. One strand of the DNA duplex is immobilized on the surface, while the other is labeled with a magnetic particle. The binding forces of the DNA duplex are measured in the absence and presence of the drug molecule, denoted as F_1 and F_2, respectively. The differential binding force (F_2-F_1) characterizes the influence of the drug–DNA binding on the stability of the DNA. The binding forces are obtained by using the FIRMS technique, as reported earlier.[18]

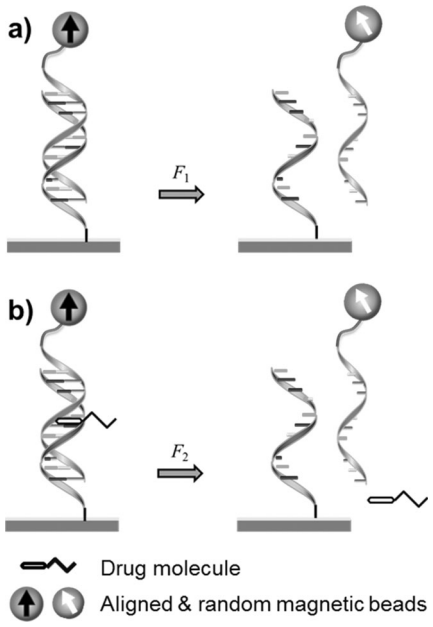

Figure 1. Concept of using differential binding force to quantify the binding specificity between a drug molecule and DNA. The difference is defined as the DNA binding force without the drug (a) subtracted from the binding force with the drug (b), namely, F_2-F_1.

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[**] Support from the US National Science Foundation under grant ECCS-1028328 is acknowledged. This work was also supported in part by the Texas Center of Superconductivity at the University of Houston. We thank Prof. Yuhong Wang for access to her laboratory and Te-Wei Tsai for technical assistance.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201407093.

The first experiment to validate this concept was carried out with the T-Hg-T system, where T = thymine and Hg = Hg^{2+}. This system has been well studied by NMR spectroscopy as well as other spectroscopic techniques.[4,6,20–22] It is thus well-known that Hg specifically intercalates into a DNA duplex at the T-T mismatching pair. Previous results show that the binding of T-Hg-T is weaker than that of C-G, but stronger than A-T.[3,23]

Our FIRMS results of the T-Hg-T system are shown in Figure 2. The concentration of Hg^{2+} in this study was 10 μM. The DNA sequence used for Hg binding was:

5′-CCC GGG TTT CCC-3′
3′-GGG CCC AAT GGG-5′

which contains a T-T pair (underlined). Further details of the experimental conditions are provided in the Supporting Information (typical magnetic signals before and after

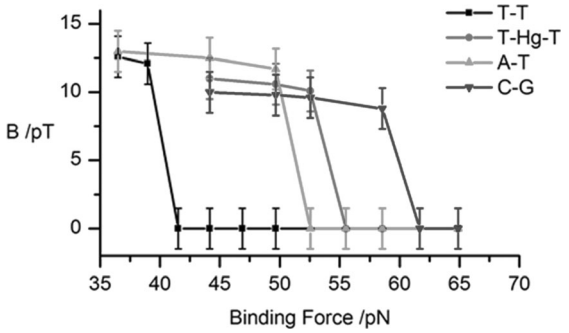

Figure 2. Measurements of the binding forces of DNA duplexes containing a T-Hg-T bond, T-T, A-T, and C-G pairs.

dissociation of the DNA duplex are included in Figure S1 in the Supporting Information). The error in the magnetic signals in the experiments carried out in this study is usually ± 1.5 pT (shown in Figure 2). The dissociation forces were calculated from the buoyant mass of the magnetic particles, the centrifugal speed at which the dissociation occurred, and the radius of the centrifuge.[18,24] Each dissociation force reported herein was calculated at least three times, with standard deviations less than 2 pN (see Table S1 in the Supporting Information). The binding force of the DNA duplex was determined to be 41 ± 1.7 pN. Upon binding of the DNA duplex with Hg, the binding force increased to 55 ± 1.7 pN, which is a 14 pN increase. For comparison, we replaced the T-T pair in the duplex with A-T and C-G. Their binding forces were measured to be 52 ± 1.7 and 59 ± 1.7 pN, respectively. The differential binding forces are thus 11 pN for A-T and 18 pN for C-G in this particular DNA system. The results are consistent with AFM results, which gave 9 ± 3 pN for A-T binding and 20 ± 3 pN for C-G pairing.[25]

Comparing the differential binding forces leads to the following binding order: A-T < T-Hg-T < C-G. This trend is consistent with the order of the melting points in the literature.[3,23] Therefore, the results validate the application of differential binding force for characterizing the binding between small molecules and DNA duplexes.

Two drug molecules have been tested for their binding selectivity (see Figure S2 in the Supporting Information for their structures). One was daunomycin, a commonly used anticancer drug. It preferentially binds to specific triplex sequences in DNA duplexes.[5] Intercalation is usually considered as the binding mode, although minor-groove binding has also been discussed.[26] However, the specificity has not been quantified in terms of the binding strength. To measure the binding forces of DNA duplexes intercalated with daunomycin, we chose 5′-CCC AAT CGA CCC-3′ as the target DNA strand, and the probe DNA was the complementary DNA strand, 5′-GGG TCG ATT GGG-3′. The duplex is designated as DNA_1. Based on previous reports, daunomycin could specifically bind to the CGA segment (underlined).[27,28] As a control experiment, we measured the differential binding force of daunomycin with a different DNA duplex (DNA_2) with the sequence 5′-CCC GGG TTT CCC-3′ and its complementary strand. Thus, DNA_2 does not contain a CGA segment. Note DNA_2 is the same as the A-T duplex in Figure 2.

The results are shown in Figure 3. The concentration of daunomycin was 100 μM. Upon intercalating with daunomycin, the binding force of DNA_1 increased from 44 ± 1.1 pN to 58 ± 1.7 pN. In contrast, no significant force difference was observed in the control experiment with DNA_2: the binding force was 52 ± 1.7 pN for the DNA and 49 ± 1.7 pN for the daunomycin–DNA_2 complex (see Table S1 in the Supporting Information). The results are consistent with the literature reports that daunomycin specifically targets the CGA sequence. In addition, we measured the signal amplitudes at various daunomycin concentrations and observed the saturation concentration to be approximately 50–75 μM (see Figure S3 in the Supporting Information). This is consistent with the saturation concentration of 40–60 μM revealed by second harmonic generation[5] and 50 μM by scanning force microscopy.[29] Several methods have been reported in the

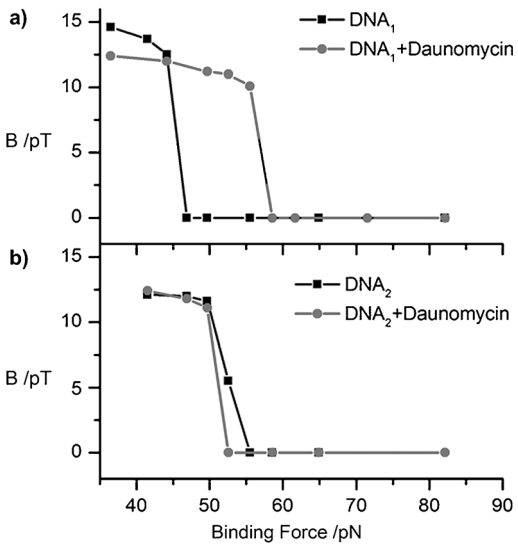

Figure 3. DNA sequence specificity in daunomycin binding. a) Specific binding of daunomycin with duplex DNA_1. b) Nonbinding between daunomycin with the DNA_2 duplex.

literature to extract the binding equilibrium constant K.[5,29,30] By using the method reported in Ref. [5], we estimated K to be $0.5–1.0 \times 10^5\,\text{M}^{-1}$, which is on the same order of magnitude as the literature values obtained from different DNA duplexes[5,29] (see the Supporting Information).

It is interesting to compare the effect of daunomycin binding with its specific DNA sequence with the binding force contributions of a single A-T or C-G pairing. The differential binding force of 14 pN shows that the contribution of daunomycin intercalation to the thermal stability is greater than that of an A-T pairing, but smaller than that of a C-G pairing.

The other drug molecule under study was tetrahydropalmatine (THP). THP is a natural alkaloid racemate extracted from *Rhizoma Corydalis*.[31] Racemic THP is included in the active compounds that have the antitumor effect of *Corydalis*. Although gas chromatography has shown that racemic THP binds enantioselectively to DNA, the two features of D-THP and L-THP binding with the DNA-immobilized column were largely unresolved in the chromatogram.[32] In addition, investigations of the interactions between THP and DNA duplex are rare. It remains unknown whether the binding force of the DNA duplex will increase after incubation of the alkaloid with DNA, which may be valuable for understanding the antitumor activity of the drug molecule.

We investigated the binding of THP with the two DNA sequences used in the previous daunomycin experiment. Both D- and L-THP were studied with each DNA sequence. The concentration of THP was 100 μM. For DNA_1 (Figure 4a), the binding of D-THP led to the binding force of the duplex increasing from 44 ± 1.7 pN to 53 ± 1.7 pN, which produces a differential binding force of 9 pN. This value shows that effective binding took place between DNA_1 and D-THP, with a binding strength similar to that of A-T pairing. Conversely, DNA_1 with L-THP did not yield an increase in the binding force, with the binding force being essentially the same as the duplex without the drug molecule. In other words, either no significant binding occurred between DNA_1 and L-THP as evident by our force spectroscopy method, or the complex cannot be mechanically resolved from the original DNA duplex.

Interestingly, the behaviors of the two chiral molecules towards DNA_2 were opposite to their binding with DNA_1. The binding force of the D-THP–DNA_2 complex was 50 ± 1.7 pN (Figure 4b), which is essentially the same as that of the DNA alone. However, the binding force of the L-THP–DNA_2 complex increased to 65 ± 1.7 pN, which is a substantial increase. The differential binding force of 13 pN is similar to that of A-T pairing.

Therefore, mutual selectivity of a DNA sequence and drug chirality is experimentally observed in the above THP–DNA systems. Elucidating the origin of this phenomenon could be of great interest to pharmaceutical research. However, more studies using other techniques and theoretical investigations are required for this important issue. Nevertheless, our high-resolution FIRMS technique is capable of determining the selectivity through the precise measurement of differential binding forces.

The differential binding forces of the various drug–DNA systems reported herein are summarized in Figure 5. This map clearly shows the mutual selectivity between DNA and drug molecules. For example, DNA_1 binds with both daunomycin and D-THP, with the former being slightly stronger than the latter, whereas it does not bind significantly with L-THP. In complicated cases, it might be common to have many similar drug structures and multiple potential DNA targets. The map of differential binding force will provide a clear picture regarding the binding selectivity.

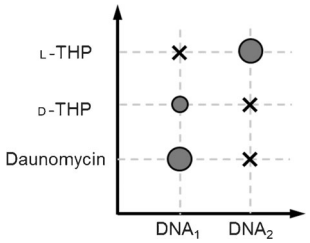

Figure 5. Mapping of differential binding forces for two DNA sequences with intercalating drug molecules daunomycin and D,L-THP. Cross: zero differential binding force; circle: positive differential binding force, with the area indicating the relative amplitude.

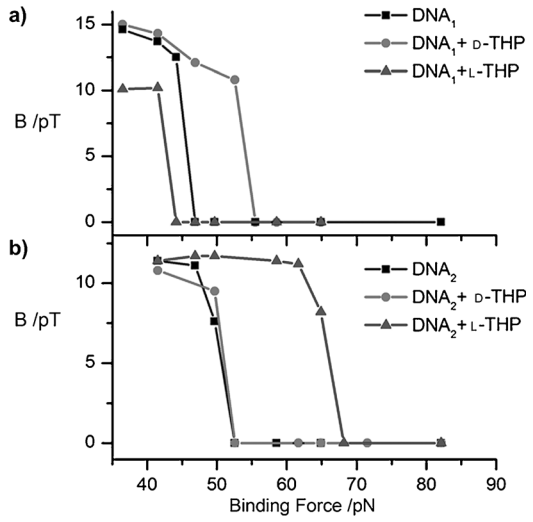

Figure 4. Sequence specificity and chiral selectivity of DNA intercalating with THP. a) DNA_1 selectively binds with D-THP. b) DNA_2 selectively binds with L-THP.

The use of differential binding force is not limited to drug–DNA systems. It will also be valid for general ligand–receptor systems involving drug molecules. The binding of the drug molecule will alter the effectiveness of the ligand–receptor recognition. Furthermore, the differential binding force does not have to be positive; a negative value will indicate reduced binding affinity between the ligand and receptor molecules. Possible examples will be inhibitors, which are designed to block the receptors from their corresponding ligands. Studies on this type of systems are currently ongoing.

Compared to other well-established force spectroscopic techniques,[33–35] such as AFM and optical tweezers, our FIRMS method offers two unique features for studying

drug–receptor interactions. One is that FIRMS measures a large number of noncovalent bonds (typically 10^4) at the same time, rather than a single bond. Therefore, it is highly efficient. The other is that the force resolution is approximately 2 pN. This allows the effect of drug molecules on ligand–receptor binding to be completely resolved, which is typically 10 pN as revealed herein. However, one limitation of our method is that it does not provide detailed conformational information on the large molecules during stretching.

In conclusion, the differential binding force provided by high-resolution FIRMS is a potent method for drug screening and other applications involving noncovalent molecular binding. The sequence specificity of Hg^{2+} and daunomycin was revealed by force spectroscopy for the first time; the results are consistent with other techniques. The D,L-THP molecules showed a novel mutual selectivity with two DNA sequences. One particular useful feature of our approach is that the small molecules under study are not labeled. This might be of high value in practical applications because of the difficulty of labeling small molecules and the consequent interferences of the labeling groups.

Experimental Section

Magnetic particles M280 were used to label one strand of the DNA duplexes. The particles have been well-characterized in previous studies.[16] After an initial magnetization by a permanent magnet, the magnetic signal of the particles was detected by an atomic magnetometer, with a sensitivity of 200 $fT\,Hz^{-1/2}$. Sample wells ($4\times2\times1\ mm^3$) with a biotin-coated bottom surface were used to immobilize the other strand of the DNA duplexes. Mechanical forces were applied using a centrifuge (Eppendorf 5417R). Detailed experimental conditions are provided in the Supporting Information.

Received: July 11, 2014
Published online: October 21, 2014

Keywords: chirality · DNA recognition · drug screening · force spectroscopy · noncovalent interactions

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