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RESEARCH ON FORCE INTERACTION BETWEEN PROTEIN AND DNA

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Introduction

Nowadays the need to design nanoscale, sensitive and flexible bio-sensors keep increasing, one of the essential challenges on this objective is the detection of binding specificity and strength between bio-molecules, which requires a thorough understanding of the mechanism governing binding interaction between bio-molecules. Research on force interaction between the complimentary pair of bio-molecules or functional groups contributes to implement the above objectives.

This research aims at the measurement of binding forces between human thrombin and its complementary DNA aptamer, which is an example of protein/DNA interaction that happens in many genetic and metabolizing processes. Human thrombin is a catalytic enzyme which catalyzes the conversion from fibrinogen to fibrin so as to facilitate blood coagulation process [1]. Aptamers are small (15 to 40 nt) DNA/RNA strands with affinities for proteins and other large biomolecules. The specific single-stranded DNA aptamer that binds to thrombin has a hairpin structure with the specific base sequence $GGNTGGN_{2-5}GGNTGG$, known as G-quadruplex. Thrombin aptamer can fit into thrombin heparin binding site and has high binding specificity with thrombin [2].

The objective of this research is to confirm the highly specific bonding between thrombin and its aptamer and measure the binding strength between the pair through Atomic Force Microscopy, which is widely applied in studies on force interaction between bio-molecules and micro/nano structures.

Method and Materials

In order to implement binding force measurement between the complementary pair via AFM, thrombin is immobilized to the AFM tip and aptamer is attached to the gold substrate. The functionalized AFM tip with thrombin then is brought into contact with aptamer to establish a ligand/receptor bond, and then pulled away at a controlled rate to subsequently break the bond. The measurements of this bond break force are performed a large number of times in order to obtain a statistically significant measure of the force interaction.

In order to functionalize AFM tip with thrombin, first incubate the probe in 2mM Mercapto-Hexadecanoic Acid (MHA) solution for 1hour, then incubate the probe in 10 mg/ml carbodiimide (EDAC) solution for 30 minutes, after that incubate the probe in 1mg/ml thrombin/PBS solution for 90 minutes, finally wash the probe with PBS and DI water for several times.

In order to coat thiolated aptamer onto gold substrate, first prepare binding buffer (20 mM Tris-HCl pH 7.4, 140 nM NaCl, 5 mM KCl, 1 mM CaCl2, 5 mM MgCl2, and 5% glycerol (w/v) in ddH_2O), then heat the 1mg/ml thiolated aptamer solution with the specific sequence 5'-HS-GCC TTA ACT GTA GTA CTG GTG AAA TTG CTG CCA TTG GTT GGT GTG GTT GG-3' in the binding buffer to 55℃ and stir for 30 seconds, after that deposit the aptamer solution onto gold substrate (25nm thick) for 30 seconds, finally pure off the excess aptamer solution and rinse the sample with ddH_2O several times .

Force curve experiments were performed via Dimension 3100 AFM in binding buffer at two nominal displacement rates (200nm/s and 2000nm/s) to test loading rate effect. 4000 force curves were taken to construct force spectrum. Since the last binding event is more likely to have fewest bond breakages, only that data were used for analysis.

Results

The typical force curve of thrombin-aptamer pair is shown in Fig.1(a). Discrete drops in forces have been suggested to be associated with unbinding interactions between thrombin and aptamer. The stepwise trend and large magnitude of binding forces show the specific binding between thrombin and aptamer. To further confirm the binding specificity, poly A DNA strands and

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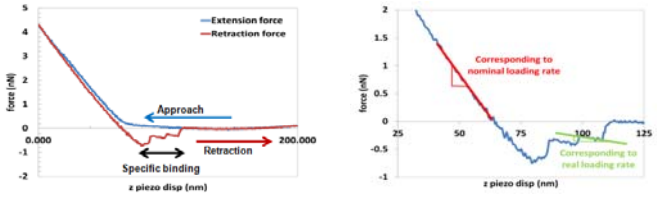
poly(ethylene) glycol (PEG) were also utilized to interact with thrombin as a comparison, the results of those experiments exhibit non-specific binding and non-binding, which validate the specific binding between thrombin and aptamer [3]. Since the nominal loading rates corresponding to nominal displacement rates didn't reflect the real loading rates before the last unbinding event happened, as shown in Fig.1(b), the real loading rates were computed for all data and then the rupture forces are categorized nto 4 different loading rate regions.



(a) Specific binding (b) Real loading rate

Fig.1 Typical force curve of thrombin-aptamer pair

The last rupture force distributions according to different loading rates are shown in Fig.2. The last rupture force spans a very broad range, which means most last bind forces comprise of several multiples of the "single-bond" binding. By picking up the smallest rupture force within each loading rate region as the force quantum, and assumed the other rupture forces are several multiples of the force quantum, the normalized binding force distribution can be obtained as Fig.3. All distributions of binding force exhibit a very sharp peak centered at the force quantum, which is a good indication of the accuracy of force quantum value.

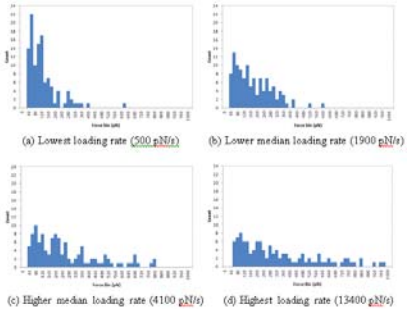


Fig.2 Last rupture force distribution

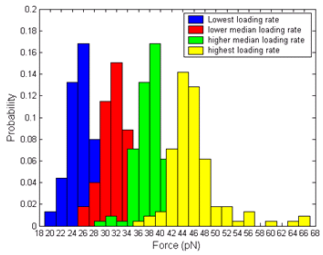


Fig.3 Normalized last rupture force distribution

The single energy barrier model indicated the binding force distribution between biomolecules as a function of loading rate shown follows Eqn.1 [4].

$$P(F_{non}) = \frac{e^{F_{non} - \frac{(e^{F_{non}} - 1)}{r_{f\_non}}}}{r_{f\_non}} \quad (1)$$

By fitting the experimental results to this model, as shown in Fig.4, we found a very good agreement between each other, which implies that the single energy barrier model is sufficiently accurate to illustrate the binding force distribution for thrombin-aptamer pair. Then by picking up the force corresponding to peak distribution value of each loading rate region, we obtain the linear dependence on binding force with respect to logarithm loading rate, as shown in Fig.5, which explicitly demonstrated the binding strength and loading rate effect of thrombin-aptamer pair.

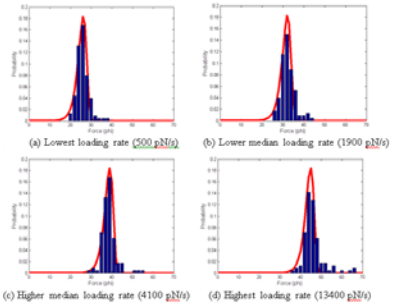


Fig.4 single energy barrier model fitting curves

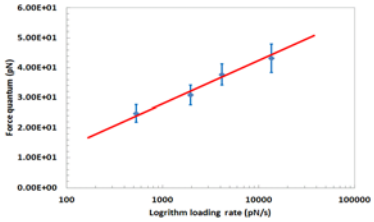


Fig.5 Binding force as a function of loading rates

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*Presenting author: Name of Author-1