Investigating the Interaction of α –Thrombin and the Thrombin-Binding DNA Aptamer

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Overview

Purpose

- Study the binding interaction of α–thrombin with a 15-mer DNA aptamer (known as the thrombin-binding aptamer) that folds into a stable G-quadruplex in the presence of monovalent cations
- Use hydrogen/deuterium amide exchange to observe the global changes in the structure of the protein as it binds the aptamer

Results

- CD spectra suggest that aptamer correctly folds into the quadruplex structure
- Global kinetic HDX experiments, reveal that the amide hydrogen atoms of holo–thrombin (i.e., protein bound to the aptamer) are more protected from HDX than those of apo–thrombin

Introduction

- α–thrombin is a serine protease found in the plasma of human blood that initiates the physiological cascade that ultimately produces a blood clot[1]
- The thrombin-binding aptamer (5'-GGT TGG TGT GGT TGG-3') was combinatorially selected for its affinity to thrombin[1] and is known to adopt an anti-parallel, chair G-quadruplex structure in solution[2], which is thought to be responsible for its binding affinity to thrombin
- In this poster, we report on the global kinetics of the DNA – thrombin interaction using H/D exchange chemical foot printing techniques

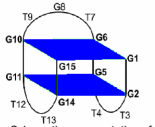
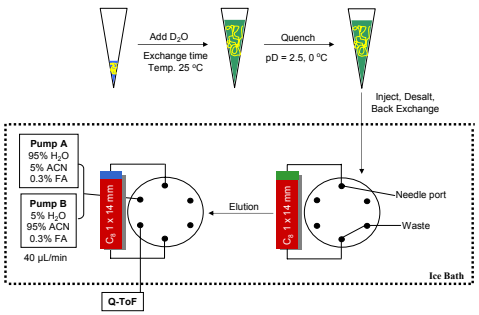


Figure 1. Schematic representation of G-quadruplex of thrombin-binding aptamer

Materials and Methods

- α-Thrombin was received from Haematologic Technologies, Inc. (Essex Junction, VT) in a 50% glycerol (*aq*, *v/v*) solution and was dialyzed against a HEPES buffer (pH 7.4, 10 mM) solution containing 140-mM NaCl, 4 mM KCl overnight
- The thrombin-binding aptamer was received from Integrated DNA Technologies, Inc. (Coralville, IA) and was used without further purification
- The thrombin-binding aptamer was annealed to promote quadruplex formation and stored at 4 ºC until analysis. The annealing was accomplished by
  - Heating solution to 95 ºC for 10 min
  - Chilling solution on ice for 20 min
- Solutions of apo-thrombin (20 μM) and holo-thrombin (20 μM protein, 300 μM aptamer) were prepared in HEPES buffer (pH 7.4, 10 mM) with 140-mM NaCl, 4 mM KCl
- All mass spectra were obtained on a Micromass Q-ToF Ultima mass spectrometer equipped with an ESI source
  - Capillary voltage was 3.40 kV, the cone voltage read back was 102 V
  - MS profile was set to scan the quadrupolar field from *m/z* 500 to *m/z* 1000 for 45% of the scan time, to dwell at *m/z* 1000 for 5% of the scan time, and to scan from *m/z* 1000 to *m/z* 2000 for 50% of the scan time
- The centroid of deconvoluted peak was used to measure extent of HDX

H/D Exchange Protocol

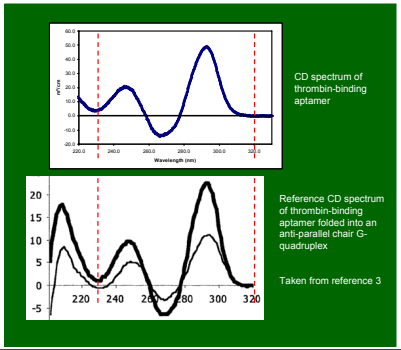


Results

Circular Dichroism (CD) Data

- If G-quadruplexes adopt the chair topology with anti-parallel strands, they have a characteristic CD spectroscopy signature[4]
- CD spectrum of annealed aptamer solution is very similar to the literature spectrum of this aptamer folded into the anti-parallel, chair G-quadruplex
- In the literature spectrum, the thicker line indicates an annealed aptamer/KCl solution while the thinner line indicates an annealed aptamer/NaCl solution

Figure 2. Comparing CD spectra of annealed aptamer solution prior to incubation with thrombin and literature spectrum of properly folded G-quadruplex

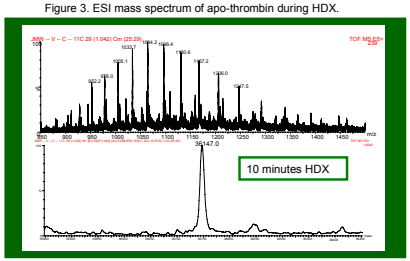


Results

The first steps in measuring the affinity of thrombin and the aptamer is to establish the suitability of the system for ESI and to determine the kinetics of hydrogen/deuterium exchange (HDX). If the kinetics show different extents of HDX at a specific time, that time can be chosen for the PLIMSTEX experiment[4].

Acceptable ESI mass spectra of apo–thrombin can be obtained after forward HDX occurred for 10 minutes (Figure 3).

Figure 3. ESI mass spectrum of apo-thrombin during HDX.



The kinetics of the exchange were measured by following the extent of exchange as a function of time for apo-thrombin and for thrombin complexed with the aptamer. There is a significant difference in the extents of exchange at all time points, indicating the greater stability (more H-bonding of amides) in the holo structure. Fitting these kinetic data with our model shows that there are many more fast-exchanging amides in the apo form than in the holo form (Figure 5). The difference indicates that a PLIMSTEX experiment should be successful.

Figure 4. Extent of deuterium uptake for apo and holo–thrombin as a function of time.

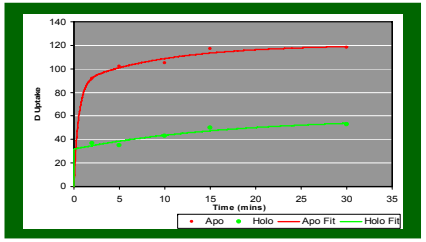
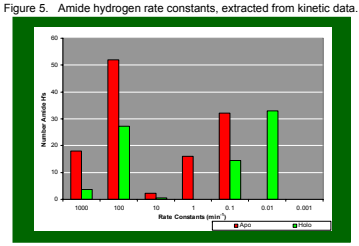


Figure 5. Amide hydrogen rate constants, extracted from kinetic data.



Conclusions

- CD spectroscopy revealed that the aptamer is correctly folded into the desired G-quadruplex having an anti-parallel chair topology
- H/D exchange protocol using an in-line guard column for desalting and back-exchange works well with this protein
- The extent of deuterium uptake by holo–thrombin is significantly less than the extent of deuterium uptake by apo–thrombin
- The large difference in the extent of deuterium uptake for apo–thrombin versus that of holo–thrombin suggests that this system is amenable to study by our recently developed PLIMSTEX method

Future Directions

- Apply the PLIMSTEX method to the interaction of thrombin with its DNA aptamer. We anticipate that the PLIMSTEX experiments will successfully measure the binding affinity of this protein/DNA interaction
- Investigate the effect of ligand binding to thrombin at the peptide level (local kinetics) by digesting the protein after H/D exchange.

References

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Acknowledgments

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