CALIFORNIA STATE SCIENCE FAIR
2015 PROJECT SUMMARY

Name(s)

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Project Number

S0528

Project Title

HDX Mass Spectrometry Study to Identify a Key, New Thrombin-TM Complex Mechanism Used to Prevent Blood Clotting

Abstract

Objectives/Goals

Blood clotting plays a vital role in the functioning of the human body. One of the most important proteins involved in this pathway is thrombin. When activated, thrombin is able to initiate coagulation and promote clot formation. However, when it forms a complex with thrombomodulin (TM), its function changes drastically to an anticoagulant, halting the coagulation cascade and preventing further clotting. Researchers don't fully understand how exactly TM alters thrombin. My goal is to gain an understanding about this complex that can help to develop better anti-clotting drugs as well as solutions to many different thrombin-induced diseases.

Methods/Materials

HDX works through the concept that the isotope of hydrogen protium (one neutron) switches with deuterium (two neutrons) when in deuterium solution. This exchange gives us valuable information regarding the structure of the protein as well as on how it folds and interacts. We can gain this information by breaking up the protein into small peptides after it exchanges in the deuterium solution and running it through a mass spectrometer.

Results

When we compare our data to another dataset, we can see differences that we never would have been able to see with just looking at the crystal structure. I can observe that TM is changing the active site of thrombin because the amount of deuterium it takes up is changing once TM binds. When we look at the active site on thrombin (also the n-terminus) on a crystal structure, we see that it is already buried in thrombin. The HDX data I collected tells us something different from what scientists previously had understood in that there is a decrease in the amount of deuterium on the n-terminus that is taken up by thrombin when thrombomodulin binds.

Conclusions/Discussion

We know TM binds on the opposite side of thrombin from the active site. The data tells us that TM is altering thrombin through allosteric changes, or changes away from the binding site. When it changes the active site, TM is changing which molecules can bind to thrombin and therefore changing its function. The data specific to the n terminus tells us that the n-terminus is going from outward to inward and that the crystal structure fails to see this movement. This new information is extremely important because the burial of the n-terminus cause serine proteases to turn into their most catalytically active state.

Summary Statement

My project used hydrogen deuterium exchange mass spectrometry to identify the key mechanism that allows thrombomodulin to alter thrombin's function when they bind and prevent it from facilitating blood clotting.

Help Received

Worked at UCSD under the supervision of Dr. Elizabeth Komives