CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

33446

Project Title

The Effect of Amino Acid Mutations on the Refolding of Thrombomodulin

Abstract

Objectives/Goals

My project was to determine if the amino acid mutations N364D, N391D, or N364D/N391D assist with the refolding of the active fragment of thrombomodulin (TM456t).

Methods/Materials

Four sets of eight fractions of TM456t were tested. Set one was TM456t with the N364D mutation, set two had the N391D mutation, set three had both mutations, and set 4 was my control and the wild type. I transformed the E. coli with my TM456t and then cultured it. I performed an inclusion body prep and resolubilized the inclusion bodies which I then loaded onto a Nickel column. I refolded the TM456t in refolding buffer and eluted it. I tested the activity and refolding success with a Protein C assay.

Results

The N364D mutation increased TM456t refolding compared to the wild type by 265%. The other mutations decreased refolding.

Conclusions/Discussion

TM456t N364D vastly improves refolding. Previous studies with refolded thrombomodulin (TM) from yeast, show that TM coated stents do not allow the body to form clots around the stents, vastly decreasing the death rate in patients with them. Studies also show that TM injections dissolve aneurysm clots. TM is not used in medicine because it can not be refolded efficiently or accurately. E. coli are an efficient and accurate way to refold TM, but TM has yet to be refolded using E. coli due to the protein#s structure. The N364D mutation allows TM to be refolded from E. coli and allows for the medical use of the protein.

Summary Statement

The refolding of the active fragment of the protein thrombomodulin by changing aspartic acid to asperigine at two seperate locations on the protein.

Help Received

Used lab equipment at University of California, San Diego under the supervision of Dr. Komives.