A Study of Troponin C Mutations A8V and D145E Related to Heart Disease

Kristin Tang, kristin.tang@gmail.com

Dr. Svetlana Tikunova, UH Department of Pharmocological and Pharmiceutical Studies

Team members: Nick Swindle, Miranda Willacey

University of Houston

Summer Undergraduate Research Fellowship

Motivation and Background

Heart failure is very common in developed countries, despite medication and surgery. It is both costly and disabling and may be lethal in some cases. Systolic and diastolic dysfunction is a common symptom in heart failure patients. Therefore, developing a methods to control the rates of muscle contraction and relaxation; and a better understanding how cardiac troponin C regulates heart function is key to treating heart disease.

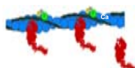
This project was conducted to compare calcium affinity and the rates of calcium dissociation between wild type cardiac troponin C and two mutants of cardiac troponin C, found in a cohort of 1025 hypetrophic cardiomyopathy patients from the Mayo Clinic*. The mutant proteins are called A8V troponin C and D145E troponin C. The purpose is to investigate how these mutations affect calcium binding properties of troponin C with the goal of applying this knowledge to treatment of heart disease.

When calcium ions bind to troponin C, cardiac muscle is able to contract. The dissociation of calcium from troponin C leads to relaxation of the muscle fibers. Troponin C forms a troponin complex by interacting with troponin I and troponin T. Troponin C consists of two globular domains that are connected by a central helix. Each domain has two EF-hand calcium binding sites. The C-terminal domain of human cardiac troponin C can also bind magnesium competitively. The mutation A8V is located at the N-terminal domain of troponin C, while D145E is located at the C-terminal domain of troponin C.

*A Functional and Structural Study of Troponin C Mutations Related to Hypertrophic Cardiomyopathy; The Journal of Biological Chemistry Volume 284 pages 19090-19100; Jose Pinto, Michelle Jones, Jingsheng Liang, Michael Ackerman, and James Potter; May 12, 2009

Figure #1

What mechanism(s) control the Ca^{2+} sensitivity AND kinetics of contraction/relaxation ???

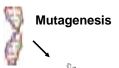


"Troponin function may be a key element"

Systems Utilized to Study Cardiac Troponin C

Mutagenesis

Troponin C



Troponin complexes

Figure #2

Location of the Mutations Within the Structure of Troponin C

Figure #3

SDS Page of Troponin C Mutants

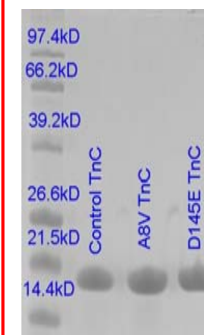
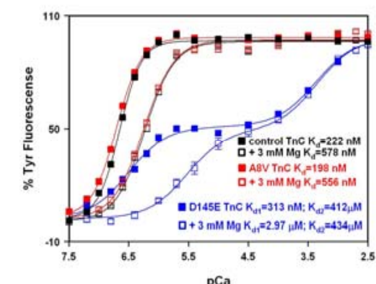


Figure #4

Determination of Calcium Affinities of Troponin C Mutants by Tyrosine Fluorescence



METHODS

Protein Isolation/Purification: Recombinant human cardiac troponin I, C, T were expressed in *E.Coli* and purified by standard laboratory procedures.

Fluorescent Labeling: TnC^{T53C} was dialyzed against 50 mM Tris, 6M Urea, 90 mM KCl, 1 mM EGTA, pH 7.5. Labeling was initiated by the addition of 3-5 M excess of IAANS. The labeling reaction was allowed to proceed in the dark for 5 hours with constant shaking at 22 ^{0}C. The reaction was stopped by addition of 2 mM DTT, and un-reacted IAANS was removed by exhaustive dialysis against 10 mM MOPS, 90 mM KCl, pH 7.0.

Steady State Fluorescence: All steady-state fluorescence measurements were performed using a Perkin-Elmer LS 55 spectrofluorimeter at 15 ^{0}C. Tyrosine fluorescence was excited at 275 nm and monitored at 303 nm. IAANS fluorescence was excited at 330 nm and monitored at 450 nm as microliter amounts of $CaCl_2$ were added to 2 mL of each Tn complex in 200 mM MOPS, 150 mM KCl, 3mM $MgCl_2$, 1mM DTT, pH 7.0. The Ca^{2+} sensitivity was reported as a dissociation constant Kd, representing a mean of 3-6 titrations. The data was fit with a single Hill equation.

Stopped Flow Fluorescence: Ca^{2+} dissociation rates (k_{off}) were measured using an Applied Photophysics Ltd. (Leatherhead, U.K.) model SX.18 MV stopped-flow instrument at 15 ^{0}C. The IAANS emission was monitored trough a 510 nm band-pass interference filter (Oriel (Stratford, CT), with excitation at 330 nm. Each k_{off} represents an average of at least five traces, and the data were fit with a single exponential. The buffer used in all stopped-flow experiments was 10 mM MOPS, 150 mM KCl, 3 mM $MgCl_2$, 1 mM DTT, pH 7.0.

Figure #5

SDS Page of Troponin Complexes

Figure #6

Determination of Calcium Affinities of Troponin Complexes by IAANS Fluorescence

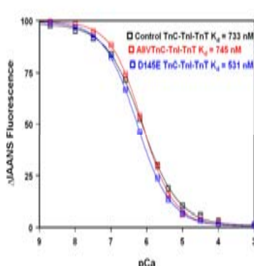
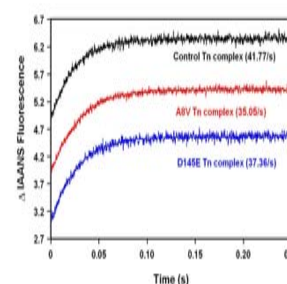


Figure #7

Determination of Calcium Dissociation Rates from Troponin Complexes by IAANS Fluorescence



CONCLUSION

Many heart failure patients experience impaired systolic or diastolic function. Troponin C regulates cardiac muscle contraction and relaxation. The mutant proteins, A8V troponin C and D145E troponin C were studied in order to gain a better understanding of how these mutants affect calcium binding properties of cardiac troponin C.

Results of the project showed that A8V troponin C-terminal domain's calcium affinity differs very little from the wild type troponin C (Figure #4). This was determined by the changes in tyrosine fluorescence when the proteins were titrated with calcium. Tyrosine reports calcium binding to the C-terminal domain. Since A8V troponin C has a mutation located at the N-terminal, its C-terminal calcium affinity is consistent with that of the control. However, D145E troponin C showed a marked decrease in calcium affinity; its K_{d1} value is approximately 1.4 times the K_d of the control troponin C while its K_{d2} value was approximately 2000 times the K_d of the control troponin C. This results from the fact that D145E troponin C's mutation is located at the calcium binding site IV at the C-terminal. It is interesting to note that D145E troponin C has two K_d values (Figure #4). When D145E troponin C was titrated with magnesium, its calcium affinity decreased, indicating competition between calcium and magnesium for binding sites. However, when D145E troponin C was labeled with IAANS in a troponin complex and titrated with calcium, its K_d value was lower than the control troponin C's K_d value by 27.6%, indicating a higher calcium affinity than the control complex (Figure #6). A8V troponin complex only showed a K_d value 1.6% higher than the control complex. IAANS reports calcium binding to the N-terminal domain of the troponin complex. The rates of calcium dissociation from the mutant troponin complexes differed slightly from the control troponin complex; calcium dissociation was 16% slower in A8V troponin complex and 10.6% slower in D145E troponin complex (Figure #8).

The fact that the rates of calcium dissociation do not differ greatly from the control troponin complex indicates that further studies need to be conducted to unveil information about the regulation of cardiac muscle contraction and relaxation by troponin C. The next step in understanding how cardiac troponin C regulates heart function is to determine calcium binding properties of cardiac troponin reconstituted into the thin filaments. Further studies in which mutant cardiac troponins C are expressed in animal muscle tissue, such as rabbit muscle, will lead to more answers.

Acknowledgements: Summer Undergraduate Research Fellowship funding, NIH Grant to Dr. Svetlana Tikunova