Expression of Sarcomeric Mitochondrial Creatine Kinase in Antarctic Fishes

Abstract
Creatine kinase (CK) plays an important role in the ATP energy cycle by acting as an energy shuttle between the mitochondrion and the site of ATP consumption. Sarcomeric mitochondrial creatine kinase (sMtCK) cDNA is found within the heart ventricle of both the icefish Chaenocephalus aceratus and the red-blooded Antarctic fish Notothenia coriiceps, but while N. coriiceps expresses CK as protein, separate studies within our lab have shown that CK protein is not expressed in Chaenocephalus aceratus. The goal of our research was to sequence sMtCK in two more fish: the icefish Chionodraco rastrosposinus and the red-blooded fish Gobiopteronthen gibberifrons. We therefore concluded that sMtCK mRNA is not necessarily representative of CK found in mitochondria.

Methods
- We isolated the DNA from the cut-out bands using a QiAquick Gel Extraction Kit.
- We cloned the isolated DNA fragments through the use of TOPO TA Cloning kit by inserting the fragment into E. coli. We selected for E. coli with our DNA fragment by treating the LB plate with ampicillin and X-GAL (Figure 3).

Results
- We used this information to create primers to measure the cDNA levels of sMtCK in the icefish C. aceratus as compared to the red-blooded fish N. coriiceps (Figure 5).

Future direction
- Due to time constraints, we were unable to measure the actual expression of sMtCK in the heart ventricles of C. aceratus and N. coriiceps.
- These studies only focused on one form of mitochondrial creatine kinase. Besides the sarcomeric form of CK, there is also an ubiquitous form of CK in mitochondria. It is possible that C. rastrosposinus and G. gibberifrons express the ubiquitous form but not the sarcomeric form of CK. It is also possible that for some reason, the primers failed to amplify the sMtCK; perhaps sMtCK exists at such a low level that we could not detect it.
- To determine whether or not C. rastrosposinus and G. gibberifrons express the ubiquitous MiCK (uMtCK), similar experiments to this one could be conducted. These experiments could use PCR with degenerate primers that bind to uMtCK instead of sMtCK.
- If uMtCK was found and sequenced in the heart ventricle of C. rastrosposinus and G. gibberifrons, quantitative real-time PCR could be performed to measure the expression of uMtCK in the heart ventricle of these fishes.

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References

Background
The sarcomeric isoform of creatine kinase (sMtCK) is an enzyme located between the inner and outer membranes of the mitochondrion; this location is thought to be important to its function in the energy production cycle of the cell (Schlattner, 2005). CK helps ensure that energy levels within cells remain stable. This is achieved by transferring the phosphate molecule from adenosine triphosphate (ATP) to creatine, which provides energy for the cell by transfer to the cytosol. The role of creatine kinase and creatine as an energy shuttle between the mitochondrion and the ATP consumer in the cytosol is thought to be important to its function in the energy production cycle of the cell (Schlattner, 2005). CK helps ensure that energy levels within cells remain stable. This is achieved by transferring the phosphate molecule from adenosine triphosphate (ATP) to creatine, which provides energy for the cell.

Methods (continued)
- We cloned the isolated DNA fragments through the use of TOPO TA Cloning kit by inserting the fragment into E. coli. We selected for E. coli with our DNA fragment by treating the LB plate with ampicillin and X-GAL (Figure 3).
- We then sent the samples to the Core Lab at the University of Alaska Fairbanks for sequencing.
- We then prepared the samples for sequencing using a Big Dye Terminator Cycle Sequencing Kit. The Big Dye reaction amplifies the DNA but randomly terminates the extension, generating fragments of different sizes.

Results (continued)
- After receiving the sequences from the Core Lab, we analyzed them and used GenBank to search for similar sequences. In theory, if our sequence closely matched with the sMtCK sequence from another organism, it would tell us that sMtCK existed within C. rastrosposinus and G. gibberifrons.
- Although our sequences aligned with other known sequences, there was insufficient coverage (< 24%) to identify our sequences as sMtCK (Figure 4).
- These results indicate that there is mostly likely no cDNA for sMtCK within the heart ventricle of C. rastrosposinus or G. gibberifrons, and therefore no expression of sMtCK mRNA.
- Our findings are consistent with studies in our lab which compare CK protein expression between icefish and red-blooded fish. These protein studies showed that no sMtCK protein was expressed in mitochondria isolated from the heart ventricle of C. rastrosposinus or G. gibberifrons.

Figure 2. Results of gel electrophoresis with G. gibberifrons cDNA (A) and C. rastrosposinus cDNA (B). The length of the ladder fragments are indicated by the numbered arrows to the left of the image.

Figure 3. (A) Normal vectors express the lacZ gene. This allows E. coli to convert X-GAL into a product, which makes the colonies appear blue. However, the insertion of the DNA fragment into the vector disrupts lacZ gene expression, causing the colony to remain white. (B) Picture of an LB plate with white and blue E. coli colonies.

Figure 4. Blast results from GenBank for C. rastrosposinus (A) and G. gibberifrons (B). Under optimum conditions, there will be red bars, as this means that there is a close match, whereas white regions within the sequence show no match at all.

Figure 5. The alignment of parts of sMtCK sequence from N. coriiceps and C. aceratus. The blue line shows a conserved splice site, the blue letters indicate the site to which the reverse primer binds, the red letters indicate the site to which the forward primer binds, and the stars show bases that match between the two species.