Functional characterization of SCP1 in Procambarus clarkii using RNA interference

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Abstract

SCP1 is an invertebrate EF-hand calcium binding protein that is hypothesized to play an important role in muscle relaxation. SCP1 is highly expressed in axial abdominal muscle (tail), and is especially abundant in fast-twitch muscle fibers. We have found three variants of the Procambarus clarkii sarcoplasmic calcium binding protein (psSCP1a, psSCP1b, psSCP1c). In this study, we used RNA interference (RNAi) to reduce the expression of all three variants and explore the function of psSCP1. We used relative quantification real-time PCR to evaluate the expression of psSCP1 in control and dsRNA injected crayfish, using variant-specific primers, primers from the non-variable region, and 18S ribosomal RNA as an endogenous control. We injected 559 bp dsRNA to reduce psSCP1 expression, resulting in an average 55% reduction of expression in comparison to controls. In individual crayfish, the amount of psSCP1 reduction varied from no reduction to 10-fold reduction compared to controls. Upon visual inspection, crayfish injected with dsRNA were found to be lethargic when compared to controls. The use of RNAi helped us visualize the functional effects of decreased psSCP1 expression, and provides a great tool for further investigation into proteins that are believed to assist in the muscle relaxation system.

Introduction

Sarcoplasmic Calcium Binding Protein (SCP) is hypothesized to play an important/significant role in muscle relaxation of fast-twitch muscle fibers, similar to the function of vertebrate parvalbumin [1,2].

The freshwater crayfish, Procambarus clarkii, has been used to study the expression of SCP and other genes that play a role in regulating calcium transport. The unique molting cycle of P. clarkii transports an extensive amount/quantity of calcium and consequently provides an excellent model to study genes involved in regulating calcium transport.

SCP has been shown to have the highest expression in the fast-twitch portions of the posterial axial muscle (tail) of crayfish. In order to understand the function of the calcium binding protein, we used RNA interference to knock down the expression of the SCP gene. Injecting double-stranded RNA blocks the expression of the SCP gene and allows one to see the effect its absence on the crayfish. Therefore, our aim was to probe the function of SCP by associating the expression of the gene with a change in movement or behavior.

Materials and Methods

Freshwater crayfish were obtained from Carolina Biological Supply and acclimated in water tanks at room temperature (23ºC) for one week. Crayfish were paired based on weight and pairs were injected with either crayfish saline (7.4PH) or dsRNA in crayfish saline (500ng/µL). Crayfish were sacrificed after 48 hours and their tail tissues were harvested and frozen at -80ºC. Total RNA was isolated using the RNA STAT-60 reagent (Tel Test “B”), and DNA was removed with Turbo DNA-free kit (Ambion). Total RNA was reverse transcribed into cDNA using random hexamers with reverse transcriptase (Applied Biosystems). The amount of cDNA was quantified using Real-Time PCR and comparing dCT values between the control crayfish and dsRNA injected crayfish.

Results

Figure 4. Effect of injected dsRNA on expression of tail SCP in P. clarkii using Real-Time PCR analysis. dCT is the difference between 18S and SCP in the number of cycles needed to amplify cDNA to a threshold value. A higher dCT value is indicative of lower SCP expression. These data are from a single batch of crayfish (n=3 for each treatment). Error bars are standard deviation.

Conclusions

• Real-Time PCR showed that dsRNA injected crayfish had decreased expression of SCP in comparison to controls. We were able to consistently decrease expression of psSCP1 by >50%, leading to a significant change between dsRNA injected individuals and control crayfish.

Future Work

• Characterize the expression of other genes that play a role in calcium transport (SERCA, PMCA, CalM) in response to a decrease in SCP expression.

Acknowledgements

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References

[1] Lexie White, Suzanne Rohrback, Christopher Gillen, unpublished data.

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