Effects of pH on Double-Stranded RNA Stability in European Corn Borer Nucleases

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Introduction

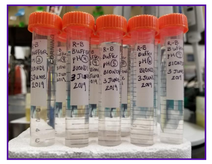
RNA interference (RNAi) is an immune response that can be exploited to make greener pesticides. It works by inciting suppression of a specific target gene using fed or injected dsRNA. Targeting a specific gene sequence also means RNAi can be used to target a specific organism. However, some insects, such as lepidopterans, have nucleases, called dsRNases, in their gut and hemolymph that sever dsRNA and lower RNAi efficiency (1). *Ostrinia nubilalis*, the European corn borer, (ECB), is a prime example of a lepidopteran pest which decimates corn supplies across the Midwest and does not respond to RNAi. Comparison of dsRNA stability in dsRNase genes in ECB and western corn rootworm (WCR), a coleopteran pest that has very high RNAi efficiency, indicates that dsRNA is rapidly degraded in ECB tissues, but not WCR tissues, despite similar expression of dsRNase genes in both species. These findings suggest that another variable, such as pH may be influencing dsRNA stability in insects (2).

Question & Hypothesis

Question: Does dsRNA degradation in ECB hemolymph and/or gut contents vary according to pH?

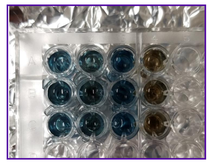
Hypothesis: DsRNA degradation will vary as pH changes, and maximum degradation will occur at the physiological pH of ECB hemolymph (pH 7.0) and gut contents (pH 8.0).

Methods



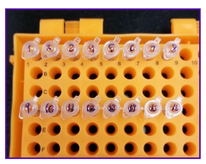
1. Formulate Britton-Robinson Buffers (3)

2. Prepare dsRNA sample

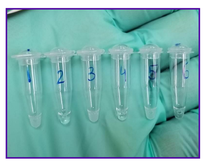


3. Quantify protein of nuclease sample

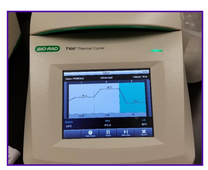
4. Normalize nuclease sample



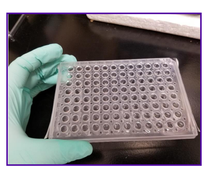
5. Incubate dsRNA with nuclease source



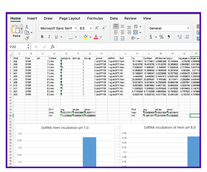
6. End the reaction with heating



7. Convert remaining dsRNA to cDNA

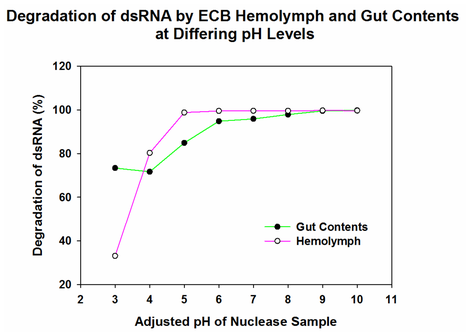


8. Quantify cDNA with qPCR



9. Calculate relative expression & analyze data

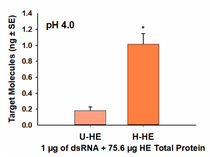
Results



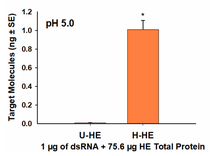
Hemolymph: Between pH 5.0 to 10.0, dsRNA was over 98% degraded in ECB hemolymph (pink line). However, from pH 3.0 to 5.0, dsRNA degradation in ECB hemolymph rapidly declined.

Gut Contents: Between pH 6.0 to 10.0, dsRNA was over 95% degraded in ECB gut contents (green line). However, from pH 3.0 to 6.0, dsRNA degradation varied between 72-96%.

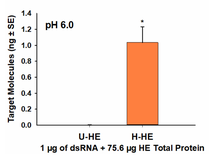
Representative Examples of Incubation Experiments in Hemolymph:



At pH 4.0, dsRNA was degraded 82% in unheated hemolymph.

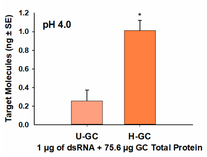


At pH 5.0, dsRNA was degraded 99% in unheated hemolymph.

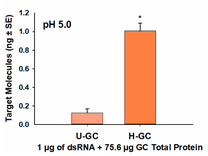


At pH 6.0, dsRNA was degraded 100% in unheated hemolymph.

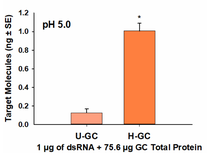
Representative Examples of Incubation Experiments in Gut Contents:



At pH 4.0, dsRNA was degraded 72% in unheated gut contents.



At pH 5.0, dsRNA was degraded 85% in unheated gut contents.



At pH 6.0, dsRNA was degraded 96% in unheated gut contents.

Discussion

DsRNA was degraded in both the midgut and hemolymph of ECB, a lepidopteran with low RNAi efficiency, which is consistent with a report that RNAi efficiency is correlated with dsRNA degradation across four insect orders (4). In addition, when pH was manipulated in migratory locust (ML) dsRNA degradation was highest in the midgut from pH 5-7.5. and much lower in the hemolymph regardless of pH (2). pH dependent degradation of dsRNA was similar in midgut tissues of ECB and ML, but between pH 4-5 dsRNA degradation decreased rapidly in ECB hemolymph, but not ML (2).

Conclusion

pH has a great effect on dsRNA degradation, and likely RNAi efficiency in ECB. DsRNA stability is increased at lower pH values and decreased at higher pH levels in both the ECB gut contents and hemolymph. However, it is also visible that nuclease activity varies between the two nuclease sources leading us to hypothesize that there are different enzymes active in the hemolymph and gut contents. In addition, variations observed between our data for ECB and previous studies in ML indicate that nuclease activity also varies among insect orders, suggesting that molecular studies on individual insects are necessary to understand RNAi efficiency.

ECB larva

ECB adults

Future Directions

Our results suggest that more research needs to be done to identify what enzymes are involved in dsRNA degradation by ECB nucleases and whether there is a strategy that can protect the dsRNA. There are several protectants that are being studied called RNAi enhancing reagents (1) so we could run this study again to determine if dsRNA stability will be increased by a protectant and whether pH will play a role in RNAi efficiency there.

Another potential direction to go in with this study is to compare the effects of pH on dsRNA degradation in WCR with ECB. It is not currently understood why RNAi efficiency is so much greater in WCR than ECB if they have many of the same dsRNases, so running this experiment with WCR could help determine just how influential pH may be in RNAi efficiency.

References

1) Cooper, A. M. W., et al., 2019. *Pest Manag. Sci.* 75(1):18-28.
2) Song H., et al. 2019. *Pest Manag. Sci.* 75(6): 1707-1717.
3) Britton, H. T. S. & Robinson, R. A. 1931. *J. Chem. Soc.:* 1456-1462.
4) Wang, K., et al., 2016. Insect Biochem. Mol. Biol. 77: 1-9.

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