Effects of Nanoparticles on Double-Stranded RNA Stability in Moth Hemolymph

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Abstract

RNA interference (RNAi) is an immune response in which double-stranded RNA (dsRNA) suppresses a target gene. By designing dsRNA to target genes that are necessary for life, dsRNA can potentially be used as an insecticide. RNAi-based insecticides are badly needed because they are more specific than conventional pesticides and because many insects have developed resistance to pesticides. Unfortunately, some insects produce enzymes that degrade dsRNA and prevent the RNAi response (Cooper *et al.,* 2018). Therefore, RNAi-based insecticides currently cannot be used to control all insects. Here we investigate dsRNA stability when incubated in hemolymph *ex vivo* to determine if degradation of dsRNA is contributing to the inadequate RNAi response exhibited by lepidopterans, such as the European corn borer (ECB, *Ostrinia nubilalis*). Our findings indicate that dsRNA is significantly degraded in ECB hemolymph, but encapsulation of dsRNA in chitosan-based nanoparticles (CB-NPs) enhances stability. These findings provide insight into RNAi efficiency limitations in insects, and may provide a method to enhance RNAi efficiency in lepidopterans and other RNAi-refractory pests.

Purpose

To determine if CB-NPs can increase dsRNA stability in ECB Hemolymph.

Questions, Hypotheses, and Predictions

Question: Can CB-NPs protect dsRNA from being degraded in larval lepidopteran (i.e., caterpillar) hemolymph?

Hypothesis: Encapsulation of dsRNA in CB-NPs increases dsRNA stability when incubated *ex vivo* in ECB hemolymph (i.e., CB-NP dsRNA will have a lower Ct value than naked dsRNA, after incubation in ECB hemolymph).

Study System

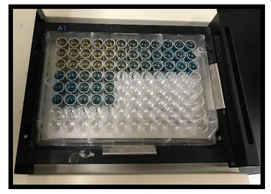
ECB, *Ostrinia nubilalis* (Lepidoptera: Crambidae) is native to Europe and invasive in North America. ECB costs farmers over a billion dollars annually in the US alone, due to yield losses and control costs (Mason *et al.,* 2017). Chemical insecticides are often ineffective against ECB because larvae escape by boring into corn stalks (Siegfried & Hellmich, 2012). For now Cry toxins (i.e., BT corn) are the most effective tool against ECB, but resistance to BT may inevitably evolve (Thieme *et al.,* 2017). Unfortunately, current RNAi-based pesticides are not available for lepidopteran pests, like ECB, because dsRNA is rapidly degraded by enzymes present in insect body fluids (Cooper *et al.,* 2018). Thus, strategies for enhancing dsRNA stability inside ECB larvae are needed.

Fifth instar European corn borer larva

Methods and Experimental Design

1) Collect ECB hemolymph samples

2) Prepare dsRNA & CB-NPs

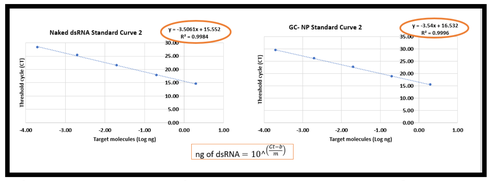


3) Quantify & normalize protein content of hemolymph samples

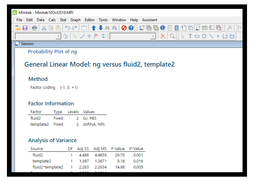
4) Incubate dsRNA& CB-NPs with hemolymph or PBS

5) Convert dsRNA to cDNA

6) Measure cDNA with RT-qPCR



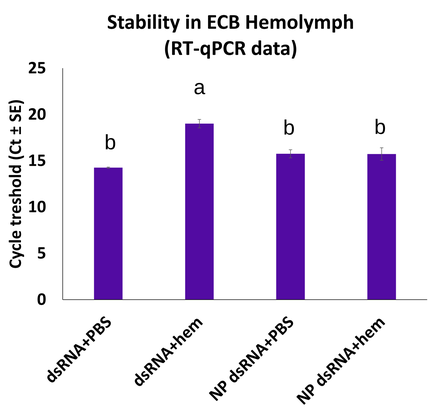
7) Convert Ct values to nanograms of dsRNA based on standard curves

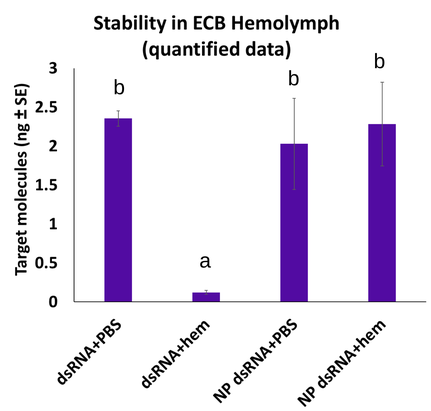


8) Analyze data with a 2-way ANOVA & Tukey Post Hoc Test

Results

DsRNA was significantly degraded when incubated in ECB hemolymph (i.e., dsRNA+hem), as compared to the buffer-only control (i.e., dsRNA+PBS). In addition, the encapsulation of dsRNA in CB-NPs significantly enhanced the stability of dsRNA when incubated in ECB hemolymph (i.e., NP dsRNA+hem). These findings indicate that nanoparticles are successful in protecting dsRNA from degradation in ECB hemolymph.





Conclusions

To solve the problem of dsRNA degradation inside the insect body, this study evaluated the effectiveness of CB-NPs for protecting dsRNA in ECB hemolymph. Our findings support the hypothesis that CB-NPs protect dsRNA from degradation in ECB hemolymph, suggesting that CB-NPs could be used to combat putative dsRNA-degrading enzymes (Cooper *et al.,* 2018). In the future, it may be possible to use CB-NPs to make RNAi more effective, both in the lab and in agriculture, so that RNAi-based insecticides and tools can be used more widely among insect orders.

Future Directions

Although this study was successful, one aspect that could be modified in the future is to not heat the samples, because the ones with hemolymph turned white and solid after quenching the incubations. The enzymes and proteins in the hemolymph basically were cooked like an egg during heating. It would be interesting to see how, or if, the results would differ if the samples were heated at a lower temperature and/or for a shorter amount of time.

Since this study shows that CB-NPs protect dsRNA from degradation *ex vivo*, next we want to determine if CB-NPs can protect dsRNA *in vivo* and enhance the lethal effects of RNAi after ECB larvae feed on CB-NP dsRNA.

In addition, CB-NPs could be tested on other destructive insect pests, such as the diamondback moth, that do not exhibit efficient RNAi responses to dsRNA.

References

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