

Introduction

Abrin, a protein toxin found in seeds of the plant *Abrus precatorius*, is a close homologue of ricin and considered a potential bioterror agent (Figure 1A). Abrin is a heterodimeric plant toxin classified as a Type II ribosome inactivating protein consisting of an enzymatic A-chain of ~30 kDa linked by a single disulfide bond to a cell-binding B chain, also of ~30 kDa, with lectin activity (Figure 1B). Abrin toxin is relevant to biodefense and law enforcement agencies due to widespread *A. precatorius* seed availability, relative ease of purification of toxin from seeds and its high toxicity. Better understanding of the physical characteristics of abrin is relevant to both the biodefense and law enforcement communities.

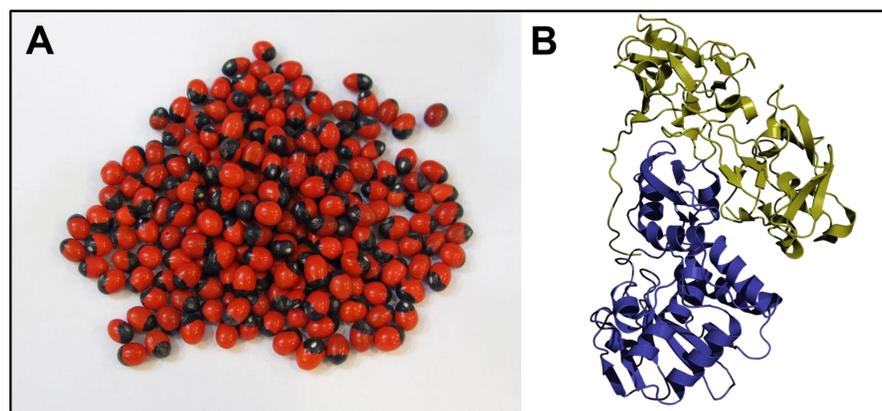


Figure 1. A) Seeds of the *Abrus precatorius* plant commonly referred to as “rosary beads” and B) Three-dimensional model of the abrin toxin quaternary protein structure. The A-chain (Purple) catalyzes the removal of an adenine in the 28S ribosomal RNA component. The B-chain (Blue) binds galactose and/or N-acetyl galactosamine residues exposed on the surface of target cells.

Target Data

The primary objective of this project was the determination of the decay rate of the toxin in water when incubated at different temperatures over time. Experiments were designed to provide the toxic half-life ($t_{1/2}$) for abrin across a temperature range of 25 to 55 °C. The $t_{1/2}$ is defined as the length of time required for the toxin to lose 50% of its original toxicity (1).

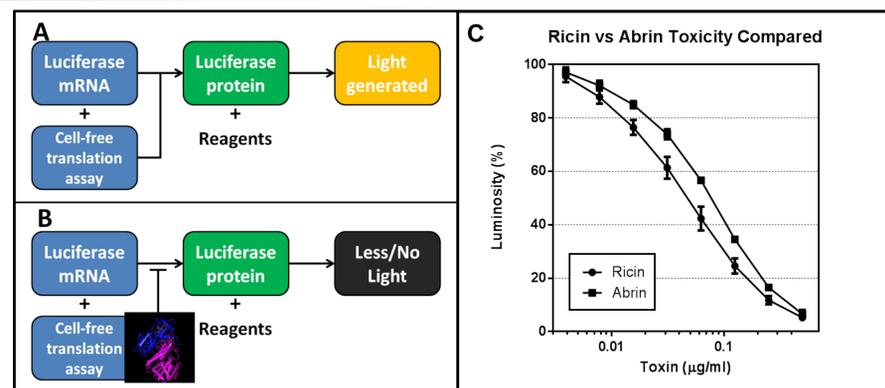


Figure 2. Outline of the *in vitro* toxicity assay and its measured output in the absence (A) or presence of toxin (B). Graph comparing toxic activity of Ricin and Abrin using the same assay (C).

Experimental approach

The American Society for Testing and Materials (ASTM) E2805 protocol, “Standard Practice for the Measurement of the Biological Activity of Ricin”, was adapted as a cell-free, *in vitro* assay for measuring abrin inhibition of protein synthesis (2; Figure 2A and 2B). The assay is an *in vitro* translation reaction that uses rabbit reticulocyte lysates as a source of ribosomes and other molecules required for ribosomal translation of RNA into protein. The toxin inactivates the ribosomes in a concentration and time-dependent manner that can be measured by the decrease in the protein product (e.g., luciferase) that is generated by the translation reaction (Figure 3).

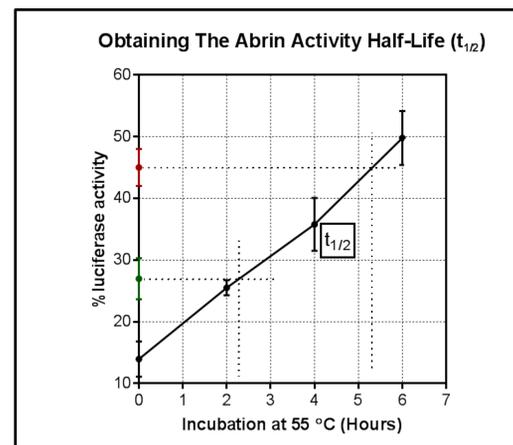


Figure 3. Toxicity decay pattern observed when Abrin is incubated at 55 °C. It is important to note that the luciferase activity is inversely correlated to toxin activity/concentration.

Results

Abrin was incubated in water at 25, 40, 45, 50 and 55 °C. When incubated in water at 25 °C (e.g., room temperature) the toxin lost activity very slowly and displayed a $t_{1/2}$ of 70-77 days (Figure 3). Plotting the $t_{1/2}$ s versus each temperature showed a non-linear trend across the temperature range tested. From approximately 25-35 °C, abrin appears to lose activity at one time-temperature dependent decay rate, while from 45-55 °C it appears to decay at a different decay rate (Figure 4A). This two-phase, $t_{1/2}$ decay trend was also observed when the toxin was incubated in an alternate buffer across the same temperature range (Figure 4B).

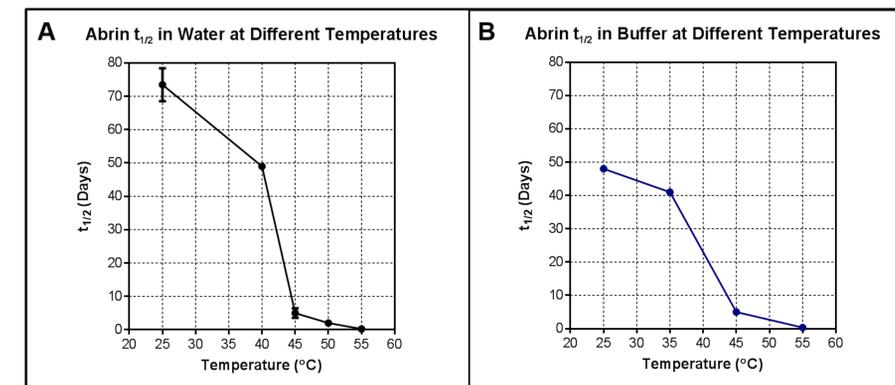


Figure 4. Graphs showing the $t_{1/2}$ values for abrin when incubated in water (A) and buffer (B) at the different temperatures shown. Each data point represents the average of 4-8 sample replicates with two technical replicates for each sample. Some error bars are present but cannot be resolved due to the scale of the Y-axis in the graph.

Conclusions

- The ASTM E2805 protocol designed to measure ricin activity has been demonstrated to be equally effective for measuring abrin activity under the stated experimental conditions.
- Abrin displays a biphasic pattern of activity loss across the temperature range employed here. This pattern was observed whether abrin was heated in water or buffer.

References

1. Jackson LS, Zhang Z, & Tolleson WH. 2010. *Journal of food science* 75(4):T65-71.
2. ASTM International. 2011. E2805 - 11 (ASTM International, <http://www.astm.org/Standards/E2805.htm>)