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Acidic vs Basic Agkistrodon Venom as an Anticoagulant

Introduction

The purpose of this investigation was to find what (if any) are the differences between the anticoagulant qualities of acidic and basic *Agkistrodon* snake venoms. The lab work done in this investigation was modeled around an experiment already done with an acidic phospholipase from *Daboia russelli* by Saikia et al to see if there is a difference between an acidic or basic phospholipase.

Discussion

The effect of an acidic phospholipase from the *Agkistrodon halys pallas* was also tested on thromboplastin. The same test was prepared on the acidic venom from *Daboia russelli*. Since the literature on acidic venoms is so much more extensive than basic venoms, the same procedure as done in the prior mentioned investigations were performed on the basic PLA2 from *Agkistrodon piscivorus piscivorus* to see if the production of thrombin would still be inhibited. In this investigation, the throboplastin produced thrombin in the presence of factor Xa, but the production of thrombin was drastically reduced when the PLA2 was added. The bands shown in **Fig. 1** reinforce this claim.

Methods

Three samples were prepared including one with just 5 µg thromboplastin, one with 1 µL factor Xa, 5 µg thrombin, and Ca^{2+} ions, and lastly one with 1 µL factor Xa, 5 µg thromboplastin, Ca^{2+} ions and the basic *Agkistrodon piscivorus piscivorus* phospholipase. These samples were then incubated for three hours at 37˚C. After being incubated, the samples were put into five separate vials. One of the vials contained 5.0 µL of reconstituted thrombin, 2.5 µL of #pH buffer and 2.5 µL of deionized water. Two of the last four vials contained 2.5 and 5.0 µL of the solution without the phospholipase, each with 2.5 µL of #pH buffer and then filled to 10 total µL by adding deionized water. The last two vials were created by using the same concentrations as without the phospholipase except that the solutions also contained

the snake venom phospholipase. All five of these vials were incubated at 37˚C for 30 minutes. The five vials and a standard were all added to a gel filled with running buffer and were allowed to run for 30 minutes at a constant 165 V. The samples were stained with imperial stain and compared against the standard.

Results

The gel shown in **Fig. 1** revealed that the production of thrombin was inhibited by the venom. The band indicating prothrombin in the trial including venom was significantly thinner and there were no bands in between prothrombin and the venom component. In the trial to its left without the phospholipase, there is a much thicker band for prothrombin and a distinct band below it for where thrombin was produced.

Conclusion

Both acidic and basic venom components cause the same slowing of production of thrombin preventing clotting. The gels from the basic *Agkistrodon piscivorus piscivorus* venom examined in this investigation produced the same banding results as from the prior literature done by Saikia et al and Wang et al, however there is still a considerable amount of work to complete. The next gel ran for this investigation should include a lane that have only the venom's PLA2, one that has thromboplastin, Ca^{2+}, and the PLA2 without the Factor Xa, and the amount of thromboplastin in its exclusive standard lane should be lessened.

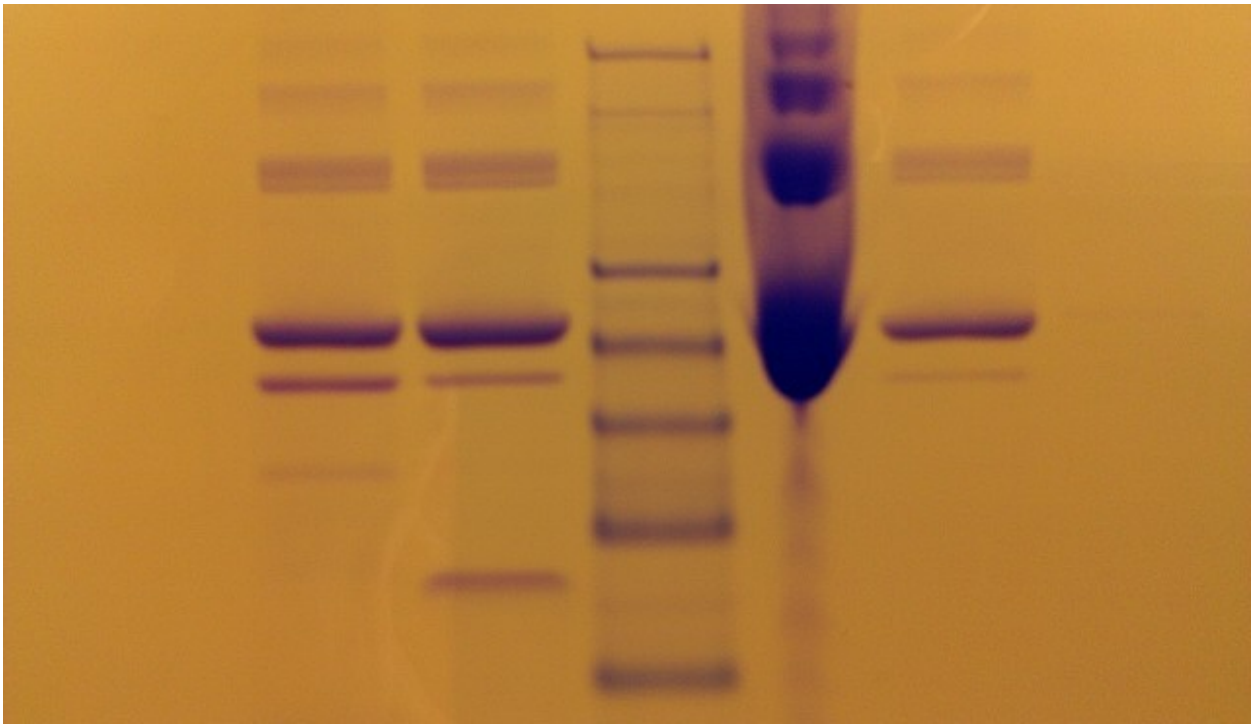

Figure 1: Lanes 1 through 6 are shown above respectively. Lane 1 shows thromboplastin, Ca^{2+}, and Factor Xa along with 2.5 µL deionized water. Lane 2 shows thromboplastin, Ca^{2+}, Factor Xa, and the venom PLA2 along with 2.5 µL deionized water. Lane 3 is the molecular weight standard. Lane 4 shows thromboplastin with nothing else added in. Lane 5 shows thromboplastin, Ca^{2+}, and Factor Xa along with 5.0 µL deionized water. Lane 6 shows thromboplastin, Ca^{2+}, Factor Xa, and the venom PLA2 along with 5.0 µL deionized water.

References

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