Comparison of Enzyme Kinetics and Inhibition of Three North American Snake Venoms

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Abstract

Baseline gelatinase activities of venom from three snake species: *Agkistrodon contortrix contortrix (Acc), Crotalus atrox (C. atrox)* and *Cerastes cerastes (Ccc)*, were measured using EnzChek® Gelatinase/Collagenase Kit E12055, and found to be similar between *Acc* and *Ccc*, while *C. atrox* venom showed lower baseline activity compared to the other two. Based on Selwyn plots of experimental data, venom from *Acc* and *C. atrox* demonstrate enzymatic stability over a wide range of substrate concentrations and reaction times, while *Ccc* venom demonstrated lower levels of stability under the same conditions. It was also found that the protease inhibitor NNGH inhibits the gelatinase activity of *C. atrox* venom more than it does for *Acc* venom. The inhibitory effect on venom gelatinase activity is not affected by enzyme pretreatment with NNGH prior to the gelatinase reaction. The DMSO used in the reaction also has an inhibitory effect, which is greater for *C. atrox* than for *Acc*. These results will be useful in understanding reaction kinetics of snake venom enzyme inhibition, which could lead to alternative treatment modalities for envenomation.

Introduction

Snake envenomation continues to be a serious problem worldwide, with numerous issues limiting treatments, including expense and availability of anti-venom throughout second and third world countries. Enzyme inhibitors as therapeutics may offer a better solution. A variety of American snake venoms were shown to have activity in sensitive protease and gelatinase/collagenase assays, and were to some extent inhibitable by a variety of protease inhibitors (Price, 2015).

Two patterns of response to hydroxamate inhibitors were found, with *C. atrox* completely inhibited and *A. c. contortrix* partially inhibited.

While the reactions in general modeled well to the Henri-Michaelis-Menton equations, inhibition did not model well to classic modes of inhibition.

It is likely that the gelatinase reaction is due to a mixture of enzymes, but it is also possible that a significant portion of the reaction with inhibitors does not follow simple classic models.

To better understand the reactions of these venoms with the gelatinase substrate, and with these inhibitors, classic descriptive enzyme reactions were conducted to confirm earlier results, redesigned to better detect reaction anomalies and directly compare reactions with these two model venoms.

Methods

As described in previous studies, the assay for collagenase activity and its inhibition was conducted in 96 well microplates with *C. atrox* venom and a fluoresceinated collagen substrate (2). Km values were estimated through nonlinear regression using GraphPad Prizm version 7 for Windows (GraphPad Software, San Diego, California, USA). Data is shown with means of triplicate determinations with standard deviations.

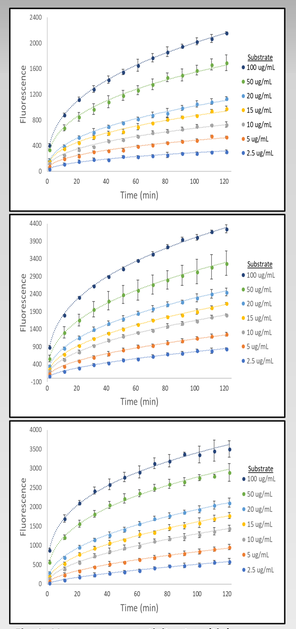

Fig. 1. Venom enzyme activity at multiple substrate concentrations for *Cerastes cerastes* (top), *Crotalus atrox (middle), Agkistrodon contortrix contortrix (*bottom).

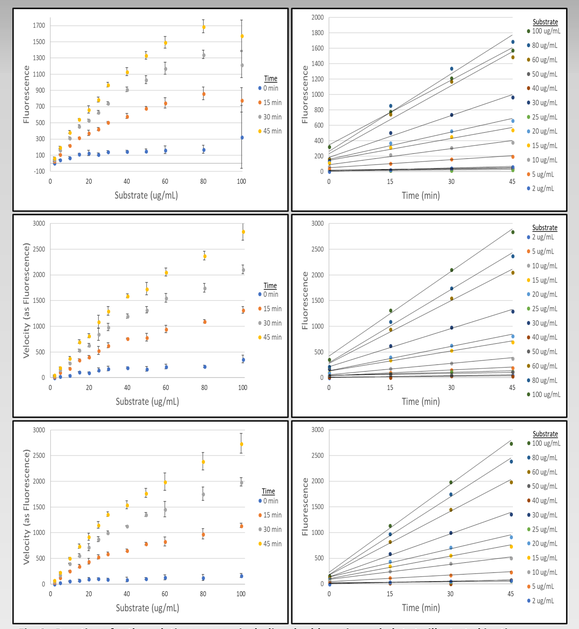

Fig. 2. Reactions for the gelatinase assay, including double reciprocal plots to illustrate kinetics. *Cerastes cerastes* (top), *Crotalus atrox (middle), Agkistrodon contortrix contortrix (*bottom).

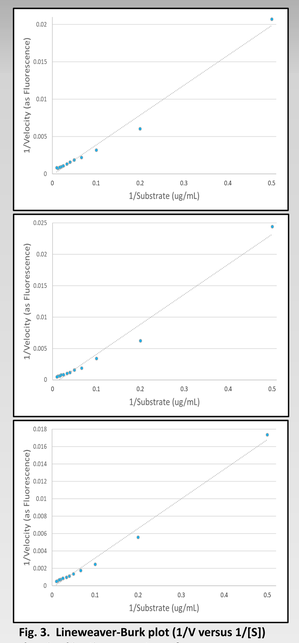

Fig. 3. Lineweaver-Burk plot (1/V versus 1/[S]) showing kinetic parameters of venom enzymes. *Cerastes cerastes* (top), *Crotalus atrox* (middle), *Agkistrodon contortrix contortrix* (bottom).

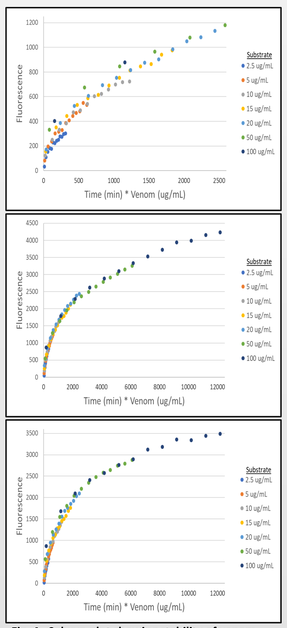

Fig. 4. Selwyn plot showing stability of venom reacting with multiple substrate concentrations. *Cerastes cerastes* (top), *Crotalus atrox* (middle), *Agkistrodon contortrix contortrix* (bottom).

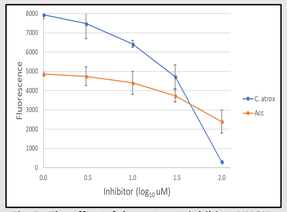

Fig. 5. The effect of the protease inhibitor NNGH on *Agkistrodon contortrix contortrix* and *Crotalus atrox* venom gelatinase activity.

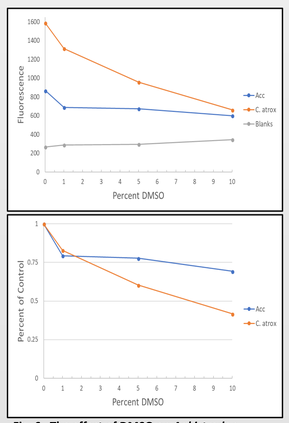

Fig. 6. The effect of DMSO on *Agkistrodon contortrix contortrix* and *Crotalus atrox* venom gelatinase activity. Substrate blank corrected data is plotted in both figures. The substrate blank data is included in the upper graph.

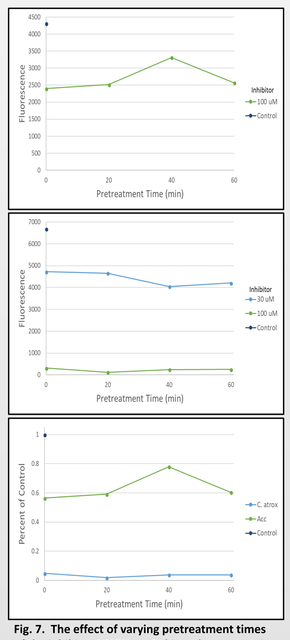

Fig. 7. The effect of varying pretreatment times of the inhibitor NNGH on *Agkistrodon contortrix contortrix (top)* and *Crotalus atrox* venom gelatinase activity (middle). Bottom panel shows this data as percent of control values for both *Acc* and *C. atrox.*

Results and Discussion

The baseline gelatinase activity of *Agkistrodon contortrix contortrix* (*Acc*), *Crotalus atrox* (*C. atrox*), and *Cerastes cerastes* (*Ccc*) venoms demonstrate ideal progress of reaction curves over a wide range of substrate concentrations (Fig. 1). This baseline activity data was analyzed using Henri-Michaelis-Menton Kinetics to calculate Km and Vmax. The Km and Vmax values were somewhat similar between *Acc* and *Ccc* venoms, however the Km and Vmax values were much higher for *C. atrox* venom (Fig. 2, 3). This indicates a lower baseline gelatinase activity in *C. atrox* venom.

- *Acc*, Km = 79 (6.4) ug/mL, Vmax = 3502 (167) ug/mL.
- *C.* atrox, Km = 124.9 (12.7) ug/mL, Vmax = 6296 (431) ug/mL.
- Ccc, Km = 76 (5.1) ug/mL, Vmax = 2630 (108) ug/mL.

Based on the Selwyn plots, the gelatinase activity for all tested substrate concentrations, as a function of time multiplied by venom concentration, follows a series of smooth reaction curves which overlap one another over a 120-minute reaction time. The close overlap between these curves indicates high enzyme stability over time for both *Acc* and *C. atrox* venom (Fig. 4). *Ccc* demonstrates relatively lower enzyme stability, as the Selwyn plot shows somewhat inconsistent reaction curves with multiple substrate concentrations. The lack of consistent curve overlap indicates that this venom has less stability over longer reaction times.

While the gelatinase activity of both *C. atrox* and *Acc* venoms is inhibited by the protease inhibitor NNGH, the inhibitory effect is greater for *C. atrox* venom than for *Acc* venom (Fig. 5). However, at higher concentrations, the inhibitory effect is greater for *Acc* venom. It was also determined that while DMSO has an inhibitory effect on the gelatinase activity of both venoms, the inhibitory effect is greater for *C. atrox* venom than *Acc* venom (Fig. 6)

In the enzyme pretreatment experiment (Fig. 7), the gelatinase activity for *Acc* and *C. atrox* venoms remained relatively unchanged as the total pretreatment time with inhibitor NNGH increases.

References

Price III, J. A. (2015, June 23). Microplate fluorescence protease assays test the inhibition of select North American snake venoms activities with an anti proteinase library. *Toxicon, 103*, 145-154. doi:10.1016/j.toxicon.2015.06.020

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