



## Serine protease from *Bothrops asper* snake venom and its inhibition in the presence of hesperitin.

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### Abstract

Our studies focus on the biochemical activity of a serine protease, named BaSP1 (*Bothrops asper* Serine Protease 1), derived from *Bothrops asper* snake venom; in the presence of hesperitin, a flavonoid obtained by the hydrolysis of hesperidin, which was obtained from citrus peel or juice. We obtained indications that hesperitin acts as an inhibitor of BaSP1. In order to evaluate the interactions between inhibitor and enzyme, circular dichroism, fluorescence spectroscopy and enzymatic assays were performed.

### Key words:

Snake venom, serine protease, hesperitin.

### Introduction

Serine proteases are among the venomous substances produced by the exocrine glands of snakes. Hemostatic changes on victims of ophidian accidents are caused by this enzyme, since it is able to cleave the Arg-Lys bonds in the  $\alpha$  and  $\beta$  chains of fibrinogen. The catalytic mechanism of the BaSPs involves a catalytic triad formed by the His 57 – Asp 102 – Ser 195 residues.

This project focuses on the study of a serine protease, called **BaSP1**, from the venom of the *Bothrops asper* snake, and in inhibiting this peptidase using hesperitin, a bioactive flavonoid from plants that was obtained by hydrolysis of hesperidin from orange peel.

### Results and Discussion

To further investigate changes in the molecular level arrangement of serine protease (BaSP1) when interacting with the inhibitor (hesperitin), fluorescence spectroscopy methods were used.

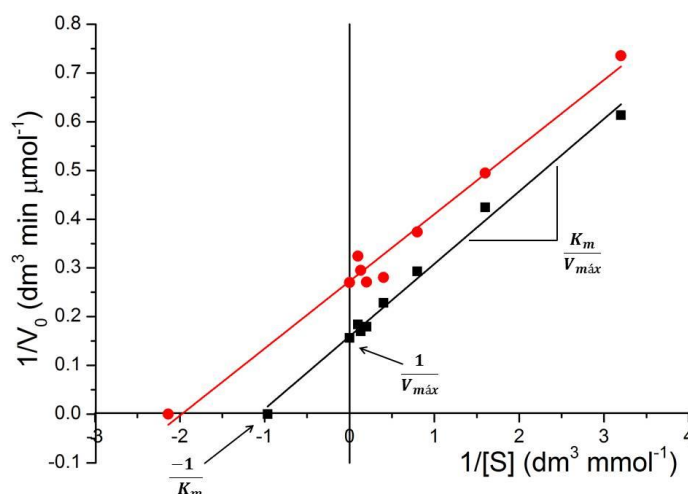
Circular dichroism spectrometry was also used to determine the secondary structure features of BaSP1.

With the enzymatic assay of target serine protease in the presence and absence of hesperitin, we sought to prove the capacity of inhibition of the flavonoid against BaSP1. Obtained data are presented in Table 1 and Figure 1.

**Table 1.** Values obtained with the Lineweaver-Burk fit of the straight lines of Figure 1:

	$K_M$ (mmol dm <sup>-3</sup> )	$V_{MAX}$ ( $\mu\text{mol dm}^{-3} \text{min}^{-1}$ )	Slope
Absence of hesperitin	1.0352	6.3981	0.1488
Presence of hesperitin	0.4684	3.7071	0.1378

According to the result obtained in circular dichroism, BaSP1 is somewhat random coil protein; and according to fluorescence spectrometry, aromatic residues (Tyr, Phe and Trp) make part of the internal structure of the BaSP1.



**Figure 1.** Lineweaver-Burk plots. The black and red lines represent the BaSP1 assays in the absence and presence of hesperitin, respectively.

### Conclusions

We have successfully purified the target enzyme, BaSP1 and characterized in biophysically and biochemically. According to our results, both,  $V_{MAX}$  and  $K_M$  changed in different proportions and, therefore, we have evidenced that hesperitin acted as a mixed inhibitor of the target enzyme.

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