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NMR in interaction studies of a serine protease from snake venom

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Abstract

In this research, we have purified a serine protease from Bothrops jararaca venom (BjSP24). This enzyme showed molar mass of 24.4 kDa as determined using MALDI-TOF. BjSP24 inhibition by hesperitin (Hst) was studied using florescence, enzymatic kinetics and nuclear magnetic resonance techinique (Saturation Transferee Difference, STD-NMR).

Key words:

Enzymes, inhibitors, NMR.

Introduction

Serine proteases are among proteins responsible for hemostatic alterations in victims of ophidian accidents. We studied a serine protease from *Bothrops jararaca*, responsible for most Brazilian ophidian accidents, in the presence of hesperetin (Hst), a bioflavonoid obtained from hesperedin's hydrolysis, which is obtained from citrus fruits' juice and peel.

The serine protease used in this study, BjSP24, was purified in two steps using HPLC ionic-exchange and affinity Benzamidine-Sepharose™ columns. In order to check purity, electrophoresis and mass spectrometry (MALDI-TOF) experiments were used.

Interaction studies between Hst and BjSP24 were evaluated applying fluorescence, enzymatic kinetics and nuclear magnetic resonance experiments.

Results and Discussion

As shown in Figure 1, BjSP24 showed an intense peak at 13.7 kDa, probably due to autolysis, and less intense peak at 24.4 kDa. It was also noted that the two steps used in the enzyme purification gave satisfactory results.

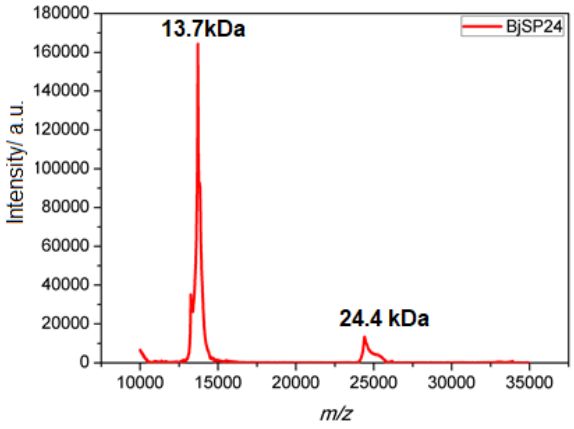

Figure 1. Target protein mass spectrum after MALDI-TOF analysis. BjSP24 showed to have 24.4 kDa.

As to verify interaction between the target enzyme and hesperetin (Hst), fluorescence experiments were performed. Using Stern-Volmer's equation, we obtained the association constant of 5.532 nmol dm^{-3} and 1.2 as the number of binding sites. Using the calculated association constant, the Gibbs energy of 46.1 kJ was calculated, which indicated that the enzyme-inhibitor interaction is not stable.

The enzymatic assay's results enabled the V_{MAX} and K_M (Table 1) calculus, using the Michaelis-Menten and the Lineweaver-Burk equation.

Table 1. Kinetic parameters for BaSP24 with and without Hst and anti-BaSP1

System	V_{MAX}/ µmol dm^{-3} min^{-1}	K_M/ mmol dm^{-3}
BjSP24	0.71 ± 0.06	2.3 ± 0.4
BjSP24 + Hst 1.0 mg cm^{-3}	0.67 ± 0.08	2.6 ± 0.7
BjSP24 + Hst 2.0 mg cm^{-3}	0.52 ± 0.05	1.7 ± 0.4
BjSP24 + Hst 3.0 mg cm^{-3}	0.56 ± 0.03	1.9 ± 0.2
BjSP24 + anti-BaSP1	0.22 ± 0.03	1.6 ± 0.6

The decrease in apparent V_{MAX} and K_M values when enzyme put at presence of Hst or immunoglobulin (anti-BaSP1) pointed to enzymatic inhibition by both entities.

The ^{1}H STD-NMR data (Figure 2) showed decreases in the intensities of the peaks at 6.90 ppm, 6.10 ppm and 3.80 ppm; which refer to the Hst 2'; 6' and 5'; and 8' and 4' hydrogens, respectively.

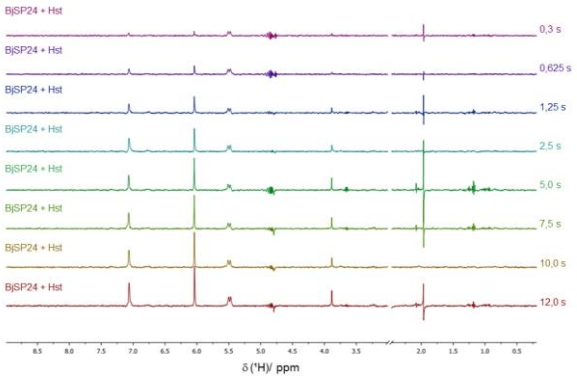

Figure 2. ^{1}H NMR (STD-NMR) spectra obtained for BjSP24 in the presence of Hst.

The difference between the 6.90 ppm hydrogen peak on- and off-resonance was plotted as a saturation time function, which enabled the dissociation constant (0.0713 s^{-1}) calculus for the target enzyme-inhibitor interaction.

Conclusions

Bothrops jararaca's serine protease was successfully purified. BjSP24 was inhibited by Hst. The interaction BjSP24-Hst is reversible and showed the association constant of 5.532 nmol dm^{-3} and the dissociation constant is 0.0713 s^{-1}.

Acknowledgements

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[1] R. Vander dos Santos, F. Villalta-Romero, D. Stanisic, L. Borro, G. Neshich e L. Tasic, *Toxicon* **2018**, *143*, 36 – 43.

[2] F. V. Romero, L. Borro, B. Mandic, T. Escalante, A. Rucavado, J. M. Gutiérrez, G. Neshich e L. Tasic, *Bioorganic & Medical Chemistry Letters* **2017**, *27*, 2018-2011.