Binding Interaction Between Thrombin and Bothrojaracin Using Surface Plasmon Resonance.

Nogueira, A. C. F.[1], Weissmuller, G.[2], Zingali, R. B.[1]

[1] Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil,[2] Departamento de Biofísica, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brasil.

The isolation and characterization of molecules with haemostatic involvement from venom of snakes seems to be valuable for the development of new target drugs. In this sense, bothojaracin isolated from *B. jararaca* venom, is a 27,000 mol. wt protein that inhibits thrombin activities. Our studies intents to characterize the binding interaction between thrombin and bothrojaracin isoforms using surface plasmon resonance, an optical method to measure the refractive index near a sensor surface (Biacore X) based on the increased resonance unit (RU) response. The venom protein was isolated by a combination of chromatographies what includes gel filtration (sephacryl S200), affinity PPACK-thrombin-Sepharose and HPLC monoQ column. After all these process, at least eight different fractions were analyzed by mass spectrometry (Maldi-Q-tof) and mascot search databases. Our results showed that most of these samples were bothrojaracin molecules separated as different isoforms. Biacore sensorgrams for the binding of thrombin (9052.3 RU immobilized) to bothojaracin at different concentracion (0.416 μM, 0.208 μM, 0.104 μM, 0.52 μM and 0.26 μM) showed differences of affinity between these identified fractions. As example of our founds, the value of the RU response for the thrombin-immobilized sensor chip surface was slightly higher for the fraction of bothrojaracin named 31 (78.5 RU) than 22 (54.2 RU), 23 (35.6 RU), 32 (73.7 RU) and 34 (54.1 RU). These findings implied that fraction 31 showed a slightly stronger binding affinity to thrombin.

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