Purification and Functional Characterization of the New Non-hemorrhagic Metalloproteinase BmajMP-II from *Bothrops marajoensis* Snake Venom

Torres-Huaco F.D.[1], Ponce-Soto, L. A.[1,2], Romero-Vargas F. F.[1], Vilca-Quispe A.[1], Huacahuire-Veja S.[1], and Marangoni, S[1].

[1]Departamento de Bioquímica, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, São Paulo, Brasil; [2]Departamento de Farmacologia, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brasil.

e-mail: huacofrank@yahoo.com.br

Bothrops snake venoms contain some metalloproteinases which do not show any hemorrhagic activities. Nevertheless, they contribute to the local effects seen after envenoming. In this work, a non-hemorrhagic metalloproteinase (BmajMP-II) was purified from *Bothrops marajoensis* snake venom.

The new metalloproteinase was isolated by a combination of gel filtration Sephadex G 75, and hydrophobic interaction chromatographies reverse phase HPLC (RP-HPLC) (µ-Bondapack C-18). The metalloproteinase was homogeneous by SDSPAGE and had a molecular mass of ~20 kDa that was unaltered by treatment with ß-mercaptoethanol. BmajMP-II shows caseinolytic, fibrinogenolytic, but no hemorrhagic activity, even with doses up to 60 µg. The caseinolytic and fibrinogenolytic activities were inhibited by EDTA, indicating that these activities were metal ion-dependent. In contrast, aprotinin, benzamidine and PMSF did not affect these activities. The caseinolytic activity of BmajMP-II had a pH optimum between 7.5 and 8.5 and was stable in solution at up to 40ºC; activity was completely lost at =60 ºC. These properties suggest that this new non-hemorrhagic metalloproteinase could belong to class P-I SVMP. This is the first report of the isolation and characterization of a low molecular weight non-hemorrhagic metalloproteinase (BmajMP-II) from *Bothrops marajoensis* venom, which may play a relevant role in blood-coating alterations which characterizes Bothrops envenomations.

Keywords: Metalloproteinase, *Bothrops marajoensis*, BmajMP-II, Caseinolytic activity, Fibrigenolytic activity, Class P-I SVMP