TITLE: EFFECTS OF METAL IONS AND PROTEASES INHIBITORS ON FIBRINOLYTIC ACTIVITY OF FIBRINOLYTIC ENZYMES FROM *Dunaliella tertiolecta*

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ABSTRACT:

Fibrinolytic enzymes are proteases with specificity for fibrin. This enzyme has pharmaceutical applications as use in the treatment of thrombosis. *Dunaliella tertiolecta* is a marine microalgae which produce those enzymes when cultured with corn steep liquor. The biochemical characterization is important to their application. The objective was evaluate effects of metal ions and proteases inhibitors on fibrinolytic activity of the enzyme purified from *D. tertiolecta.* Microalgae was grown in the f/2 medium supplemented with corn steep liquor until to reach the end of exponential phase of cell growth. The enzymes extracted from cell by homogenizing, precipitation by acetone and purification by ion exchange chromatography. To evaluate effects of metal ions, the purified enzyme was incubated during 1 h at room temperature with 5mM of various divalent metal ions such as $CaCl_2$, $CoCl_2$, $MgSO_4$, $ZnSO_4$, $CuSO_4$, and $FeSO_4$. To evaluate effects of proteases inhibitors, the fibrinolytic enzyme was exposed during 60 minutes at 37ºC with to the followings inhibitors: Pepstatin A and PMSF (fluoride-methylphenylsulfonyl $C_7H_7FO_2S$), 2-mercaptoethanol (2-hydroxy1-ethanethiol-C_2H_6SO), EDTA (ethylenediaminetetraacetic-acid) and Iodoacetic Acid. Enzyme activity measured in the absence of protease inhibitors and metal ions was considered 100%. The activity of enzyme from *D. tertiolecta* was slightly inhibited by Zn^{2+}, Cu^{2+}, Ca^{2+} and Mg^{2+}, was also partially inhibited by Co^{2+}, and was overly enhanced by Fe^{2+} (874.5%). This date indicates that the enzyme is Fe-dependent. All proteases inhibitors tested were capable of inhibit fibrinolytic enzyme from *D. tertiolecta*, except iodoacetic acid, a cysteine peptidase inhibitor which was able to activate the enzyme. PMSF, which is a serine protease inhibitor, inhibited totally the fibrinolytic enzyme. Others protease inhibitors, such EDTA and Pespstatin A, which are respectively metalloprotease and aspartic protease inhibitor, also were able to inhibit the fibrinolytic enzyme, but not like PMSF. Fibrinolytic enzyme from *D. tertiolecta* is a serine protease Fe-dependent.

Keywords: Microalgae; Fibrinolytic enzyme; biochemical characterization; thrombosis

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