Isolation and Characterization of a ß-Glucuronidase of the Mollusk *Bulimulus tenuissimus*

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Efficient enzymes for the synthesis and degradation of glycosaminoglycans are important in natural biological systems of these glycoconjugates. Therefore, an understanding of the glycosidases structures and functions is imperative towards a better comprehension of their natural role. The aim of the present work was to study the enzyme involved in the metabolism of sulfated glycosaminoglycans in the mollusk *Bulimulus tenuissimus*. The mollusk tissues were homogenized at 4°C. in a 0.1 M sodium acetate buffer, pH 5.0, then centrifuged at 20,000 x g. The proteins in the supernatant were submitted to fractioning with increasing concentrations of ammonium sulfate (0-50% and 50-80%) getting the fractions F_1 and F_2, respectively, with the best activity visualized in the fraction F_2 (50-80%), as verified by agarose gel electrophoresis carried in PDA buffer. A ß- glucuronidase (F_3) was isolated by gel filtration chromatography (Bio-gel A) - 1.5 m of fraction F_2 on flow of 1ml/3min. High Performance Liquid Chromatography (HPLC) revealed only a protein band, confirming a 1.8% yield after 170 fold purification, with molecular mass of 116 kDa determined by electrophoresis in polyacrylamide gel with SDS. The determination of a number of kinetic parameters ideal for p-nitrophenyl-ß-glucuronide catalysis by ß- glucuronidase (F_3), demonstrated that its optimal activity is at pH 5.0 and temperature of 55°C for 4 hours, with an apparent K_m of 2.68 mM. A total of 0.88 µg of ß-glucuronidase is needed for the degradation of 0.09 µM p-N-ß-glucuronide. Future studies on the three dimensional structure and the amino acid sequence will enable a deeper discussion of the inhibition and stimulation of some compounds in the ß-glucuronidase activity of the mollusk *Bulimulus tenuissimus.*

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