Determination of Relative Molecular Mass of L-asparaginase Produce by Aspergillus terreus (PC-1.7.A)

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L-asparaginase produced by bacteria (L-asparagine-aminohydrolase, EC 3.5.1.1) is an enzyme used clinically as antitumoral agent in acute lymphoblastic leukemia for more than 30 years. It converts the amino acid asparagine present in blood, which is essential to some tumor lines, to aspartic acid and ammonia. In previous work we selected an Aspergillus terreus strain (PC-1.7.A) capable to produce high levels of L-asparaginase which showed antitumoral activity against at least four cell lines. The aim of this study was to determine the relative molecular mass of this L-asparaginase by gel-exclusion and electrophoresis. The culture broth was separated from mycelium by filtration and dialyzed, concentrated and applied to an ion-exchange column (DEAE Sepharose Fast Flow). The only pool obtained was rechromatographed on a gel exclusion column (Sephacryl S-200HR). The elution volume obtained with L-asparaginase activity was 21,09 mL and its molecular mass was estimated at 136 kDa. The pool obtained from gel exclusion column was applied in gel electrophoresis and two proteins bands with 180 and 130 kDa were obtained. The band with molecular mass of 130 kDa corresponds to the molecular mass obtained by gel exclusion column, leading us to conclude that Aspergillus terreus (PC-1.7.A) produces a L-asparaginase with relative molecular mass of 136 kDa which probably is composed for a single subunit, differently those founded in the others microorganisms that present between 4-8 subunits.

Keywords: Aspergillus terreus, Chromatography, L-asparaginase

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