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Ethanol Precipitation and Characterization of L-Asparaginase from Aspergillus oryzae

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Abstract : L-asparaginase (L-ASNase) is the gold standard treatment for acute lymphoblastic leukemia that mainly affects pediatric patients; treatment increases survival from 20% to 90%. The characterization of other L-Asparaginases, apart from the most used from Escherichia coli and Erwinia chrysanthemi, has been reported, but the choice of the most appropriate is still under debate. This choice should be based on its pharmacokinetics, immune hypersensitivity, doses, prices, pharmacodynamics. The main factors influencing the antileukemic activity of ASNase are enzymatic activity, Km, glutaminase activity, clearance of the enzyme and development of resistance. However, most of the commercialized enzyme present an intrinsic glutaminase activity, which is responsible for some side effects. In this study, glutaminase free asparaginase produced from Aspergillus oryzae was precipitated in different percentages of ethanol (0–80%), until optimum ethanol concentration of 60% (w/w) was found. Following, precipitation of crude L-ASNase was performed in a single step, using 60% (w/w) ethanol, under constant agitation and temperature. It presented activity of 135.45 U/mg and after gel filtration chromatography with Sephadex G-the enzymatic activity was 322.02 U/mg. The apparent molecular mass of the purified L-ASNase fraction was estimated by 10% SDS-PAGE. Proteins were stained with Coomassie Brilliant Blue R-250. The molar mass range was from 10 kDa to 250 kDa. L-ASNase from Aspergillus oryzae was characterized aiming possible therapeutic use. Four different buffers (phosphate-citrate buffer pH 2.6 to 5.8; phosphate buffer pH 5.8 to 7.4; Tris - HCl pH 7.4 to 9.0; and carbonate buffer pH 9.8 to 10.6) were used to measure the optimum pH for L-ASNase activity. The optimum temperature for enzyme activity was measured at optimal pH conditions (Tris-HCl and phosphate buffer, pH 7.4) at different temperatures ranging from 5 to 55°C. All activities were calculated by quantifying the free ammonia, using the Nessler reagent. The kinetic parameters calculation, e.g. Michaelis-Menten constant (Km), maximum velocity (Vmax) and Hills coefficient (n), were performed by incubating the enzyme in different concentrations of the substrate at optimum conditions of pH and fitted on Hill's equation. This glutaminase free asparaginase showed a low Km (3.39 mM and 3.81 mM) and enzymatic activity of 135.45 U/mg after precipitation with ethanol. After gel filtration chromatography it rose to 322.02 U/mg. Optimum activity was found between pH 5.8 - 9.0, best activity results with phosphate buffer pH 7.4 and Tris-HCl pH 7.4 and showed activity from 5°C to 55°C. These results indicate that L-ASNase from A. oryzae has the potential for human use.

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