PROTEASE ACTIVITY FROM CRATYLIA MOLLIS SEEDS

Bezerra, Y.B.S.¹; Lima, T.A.¹; Coelho, L.C.B.B.¹; Napoleão, T.H.¹; Paiva, P.M.G.¹

¹Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife, Brazil

Introduction: Cratylia mollis (Fabaceae) is a Brazilian tree found in semiarid region, popularly known as “camaratu bean”. The C. mollis seeds are a rich source of lectins and trypsin inhibitors which have shown several biological properties. Plant proteases have been isolated and studies demonstrated their biotechnological applications.

Objective: This study investigated the presence of proteases in C. mollis seed extract, defined the isolation procedure as well as partial characterization of isolated protein.

Material and Methods: Proteins from C. mollis seed powder (10 g) were extracted using 0.15 M NaCl (100 mL), during 16 h at 25°C. The crude extract, obtained after filtration and centrifugation (9000 g, 15 min), was treated with ammonium sulfate (80% saturation). After centrifugation (9000 g, 15 min), the precipitated fraction (PF) was dialyzed against distilled water (4 h) and 0.1 M Tris-HCl pH 8.0 (4 h) and submitted to ion-exchange chromatography on DEAE-cellulose equilibrated with Tris buffer. After washing using equilibrating solution, the adsorbed proteins were eluted with 1.0 M NaCl. The extract, PF and the non-adsorbed (P1) and adsorbed (P2) protein peaks from chromatography were evaluated for protein concentration, protease activity using azocasein as substrate and polyacrylamide gel electrophoresis at presence of sodium dodecyl sulfate (SDS-PAGE). Results and discussion: Protease activity was detected in the extract (30.3 U/mg), PF (36.0 U/mg) and P1 (60.5 U/mg) from DEAE-cellulose chromatography. The adsorbed protein (P2) did not show protease activity. The enzyme was purified 2.0 times. The electrophoresis profile of P1 showed four polypeptide bands with molecular mass 152.2, 20.78, 11.23 and 7.9 kDa. Conclusion: Proteases from C. mollis seeds are cationic proteins and were isolated from extract protein mixture by cation exchange chromatography without trypsin inhibitor and lectin contamination.

Key words: Cratylia mollis, cationic proteases, enzyme isolation.

Supported by: CAPES, CNPq, FACEPE and MCTI.