PARTIAL PURIFICATION AND MOLECULAR MASS DETERMINATION OF DEOXYRIBONUCLEAESES FROM BANDURA (*Nepenthes distillatoria)*

Piumal Wijesinghe[1], E.A. Prabodha Ekanayaka[1], Sanath Rajapakse[2] and Senarath B.P. Athauda[1]

[1]*Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Peradeniya*

[2]*Department of Molecular Biology and Biotechnology, Faculty of Science, University of Peradeniya, Peradeniya*

Introduction

There are several carnivorous plants of different genera in nature, which catch prey, mainly insects and digest them by their hydrolytic enzymes and absorb the digested products as nutrients. *Nepenthes distillatoria* is one such carnivorous plant endemic to Sri Lanka, which grows, in South West and Southern parts of the island. Recently Athauda *et al.* (2004) has successfully isolated and structurally characterized two acid proteinases from the pitcher juice of *N. distillatoria.* They have reported the unusual stability of the enzymes as the striking property. Both enzymes were remarkably stable at high temperatures and over a wide range of pHs. In addition to proteinase activity, preliminary investigations by Chin *et al.* (2007) indicated the presence of deoxyribonucleolytic (DNase) activity in the crude pitcher juice of *N. distillatoria.* DNases are important analytical enzymes and have found extensive applications in molecular biology and biotechnology. Hence, further purification of DNases was carried out to determine the molecular weight of each partially purified enzymes.

Materials and Methods

The assay procedure developed by Chin *et al* (2007) for analyzing DNase activity in *N. distillatoria* was used for purification and further studies. Partial purification of DNases present in the crude juice was performed using anion exchange chromatography at 4°C. First, a 100 ml DEAE-cellulose column was equilibrated using 10 mM sodium phosphate buffer at pH 7.0. Then 1.5L of *Nepenthes* crude juice previously equilibrated with the same buffer at pH 7.0 was injected into the column. The column was washed extensively with the same buffer and the bound proteins were eluted with a linear gradient of (0 -1M) NaCl in 0.01M sodium phosphate buffer at pH 7.0. Ninety 10 ml fractions were collected using a fraction collector and assays were performed to determine the DNase activity. Fractions with identical DNase activity were pooled together. In order to determine the pH stability, enzymes were separately incubated in buffers at different pH (from pH 2.0 - 8.0) over a period of two weeks at 4°C. Aliquots were removed at 0, 1, 3, 7, 11 and 14, days intervals and remaining DNase activity of each aliquot was determined. To determine the stability at different temperature, the enzymes were

incubated at different temperatures (30°C, 37°C, 45°C and 50°C) in pH 3.0 buffer for a period of two weeks. Aliquots were removed at 0, 1, 3, 7, 10 and 14 days and the percentage remaining DNase activity in each aliquot was determined. Determination of the effect of metal ions on the DNase activity was conducted using the same assay procedure, with the inclusion of test samples containing metal ions of interest (Zn^{2+}, Ca^{2+} and Mg^{2+}). Once the metal ions that displayed an effect were identified, crude juice was analyzed using a series of standard solutions with concentrations ranging from 1 mM to 10 mM of that metal ion. Both the crude juice and the individual DNases isolated were analyzed. Concentrated, partially purified enzymes (DNase 2 and DNase 3) were used to determine the molecular masses and gel filtration chromatography was performed using 200 ml Sephacryl S200 column (flow rate was 600µl/min) and ninety five 6 ml fractions were collected. Direct absorbance of all the fractions at 280 nm (OD280) and activity of each fraction were measured. Molecular mass was determined using SDS-PAGE followed by commassie blue staining and Gel filtration chromatography on a calibrated Sephacryl S 200 column.

Results and Discussion

Partial purification on DEAE cellulose chromatography indicates the presence of four different DNA digesting enzymes (Dnase 1, DNase 2, DNase 3 and DNase 4) in the crude pitcher juice. Enzymes were eluted at NaCl concentrations, 0.2 M (DNase 1), 0.35M (DNase 2), 0.6 M (DNase 3) and 0.8 M (DNase 4). DNases 2 and 3 were found to be the abundant enzymes according to the total activity for each enzyme. All the four enzymes had a significant stability over a broad pH range for two weeks. The percentage activity remaining at pH 8.0 was around 60% and at pH 3.0 was around 75%. All the four enzymes showed a remarkable stability at 50^0C. On average, all the enzymes had more than 75% remaining activity after two weeks at 50°C and at 37°C it was more than 85%. Furthermore, it was found that the activity of the crude juice increased by about 39% in the presence of Zn^{2+} ions in the reaction mixture. The greatest enhancement of activity caused by Zn^{2+} (42%) was observed at concentration of 6 mM of Zn^{2+}. In the presence of Ca^{2+}, the activity was enhanced by about 34% and the greatest enhancement of 35% was recorded at a concentration of 4 mM of Ca^{2+}. However, the results seem to be unaffected by the presence of Mg^{2+}. Activities of all four enzymes are positively enhanced by the presence of 1mM of Zn^{2+} (DNase 1- 30%, DNase 2- 60%, DNase 3- 37%, DNase 4- 49%). Ca^{2+} at 1mM concentration also showed positive enhancement of DNase 1 (30%), DNase 2 (14%) and DNase 3 (13%). The molecular mass of partially purified DNase 2 determined by SDS-PAGE was 53 kDa and according to gel filtration it was 52 kDa. Molecular mass of the DNase 3 was found to be 37 kDa using gel filtration chromatography. These results demonstrate the stability of all the four enzymes over a broad pH range and at 37 and 50^0C. These results are comparable with the results obtained with the crude pitcher juice. Accordingly, these enzymes seem to

be resistant to the proteinase activity in the crude pitcher juice. Therefore, DNases present in the pitcher fluid of *N. distillatoria* may have remarkable properties to withstand against temperatures, a wide pH range as well as attack by proteinases. These features clearly indicate the wide applicability the enzymes. Further studies are in progress on purification and characterization of the different DNases.

References

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