

SCBC/D 3231008 : MAJOR : BIOCHEMISTRY ; Ph.D. (BIOCHEMISTRY)
 KEY WORD : HIGH PERFORMANCE LIQUID CHROMATOGRAPHY /
 MANNOSIDASE / SPECIFICITY / SYNTHESIS / *VIGNA*
UMBELLATA

PATJARAPORN WONGVITHOONYAPORN : SEPARATION,
 CHARACTERIZATION, AND SPECIFICITY STUDY OF α -MANNOSIDASES FROM
VIGNA UMBELLATA. THESIS ADVISOR : M.R. JISNUSON SVASTI Ph.D., PICHIT
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 Ph.D. 260 p. ISBN 974-589-112-6.

Two α -mannosidases (EC 3.2.1.24), I and II, were isolated from *Vigna umbellata* by dialysis against 0.02 M glycine-sodium hydroxide buffer, pH 10.0, then chromatographed on DEAE-cellulose, hydroxyapatite, CM-cellulose (NaCl gradient), Sephacryl HR S-300, and CM-cellulose (pH gradient) column. The native molecular weight of both isozymes is estimated to be 297 – 329 kDa, but pIs of form I are 5.03 – 5.34 while pIs of form II are 5.46 – 6.20. Optimal pH and temperature of both isozymes for assaying with *p*-nitrophenyl- α -D-mannopyranoside (*p*NP- α -Man) are at pH 4.2 and 60°C. The α -mannosidase activity from this source is lost after dialysis against 1 mM EDTA at acidic pH but not at neutral or alkaline pH. Zn^{2+} is the essential metal ion for its activity and can stabilise the enzyme activity when stored at acidic pH, while $FeCl_2$, $FeCl_3$, and $MnCl_2$ inhibit enzyme activity. The enzyme is stable at very broad pH and at temperature 30° – 40°C. Effect of swainsonine and 1-deoxymannojirimycin on both isozymes are similar in that both isozyme activities are completely inhibited by swainsonine but only by about 22 – 27 % by 1-deoxymannojirimycin. However, the α -mannosidase II has lower K_m than α -mannosidase I for both *p*NP- α -Man and $Man\alpha(1-2)Man$. Study of specificity of both α -mannosidases from *Vigna umbellata* reveals that these enzymes are most specific towards $Man\alpha(1-2)Man$ in both hydrolysis and synthesis. However, the hydrolytic specificity towards $Man\alpha(1-3)[Man\alpha(1-6)]Man$ of the two isozymes are different in that α -mannosidase II can produce $Man\alpha(1-3)Man$ more than $Man\alpha(1-6)Man$, but vice versa for α -mannosidase I. Variation of synthesis conditions does not change the synthetic specificity which is towards $Man\alpha(1-2)Man$. Optimal conditions for mannobiose synthesis of 0.5 U/ml *Vigna* α -mannosidase are 70 % (w/v) mannose incubated at 60°C and pH 4.0. In the presence of mannose (10 % – 80 % (w/v)) at temperature less than 50°C, pH 4.0 – 10.0, the *Vigna* α -mannosidase is more stable than without mannose.