E210 Cell Wall Acid Phosphatase from Maize Coleoptiles

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Cell wall acid phosphatase was extracted from freeze-dried maize coleoptiles with 0.1M calcium chloride and purified by the combination of DEAE Sepharose, Phenyl Sepharose, Con A Sepharose and Superdex-200 gel filtration chromatography. Enzyme activity was measured by estimating the amount of the p-nitrophenol out of p-nitrophenyl phosphate(pNPP) during the purification procedure. Specific activity of the enzyme was measured with o-carboxyphenyl phosphate as a substrate. The activity was also shown on the nondenaturing gel by the *in situ* activity staining which used 5-bromo-4-chloro-3-indolyl-phosphate and nitroblue tetrazolium as substrates. The molecular weight of the purified acid phosphatase was estimated to be 73kD based on SDS-PAGE and it appeared to be a glycoprotein. The purified enzyme displayed an optimum pH at 4.5, and its K_m was 8.6mM when pNPP was used. The purified cell walls from maize coleoptiles showed a higher activity of acid phosphatase when pNPP was used as a substrate. With other substrates such as AMP, ATP, phosphenolpyruvate and α-naphtyl phosphate, the enzyme did not give any significant activities. The enzyme was inhibited by phosphate, and about 65% of the inhibition was found in the presence of 20mM potassium phosphate. This study also shows that the total activity of cell wall acid phosphatases increased in the water-stressed coleoptiles two times higher than in the well-watered coleoptiles.

E211 Effect of Methyljasmonate and Salicylic Acid on the Antioxidant Enzymes Activity in Wounded Mung bean (*Vigna radiata* (L.) Wilczek) Leaves

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After wounding, methyl jasmonate (MeJA), and salicylic acid (SA) treatment, six enzymes activity were measured - super oxide dismutase (SOD), ascorbic acid dependent peroxidase (APX), ferulic acid dependent peroxidase (FPX), guaiacol dependent peroxidase (GPX), catalase (CAT), and lipoxygenase (LOX). In wounding experiment, FPX, GPX, and LOX activity were increased. MeJA increased the activity of GPX and LOX. Salicylic acid, however, did not affect the activity of these six enzymes. Native isoelectric focusing (pH4 - 7) was performed on vertical polyacrylamide gels. Major POX isozymes were detected at pI 5.9 and minors at pI 6.4, 5.8, 5.6, 4.7. LOX isozymes were detected at pI 5. Both wounding and MeJA had induced POX and LOX activity.