E225 Purification and Biochemical Characterization of Soluble Acid Phosphatase from Maize Coleoptiles

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The soluble acid phosphatase (APase, EC 3.1.3.2) from maize (*Zea mays* L.) coleoptiles was purified by polyethylene glycol fractionation, DEAE-Sepharose, Con A-Sepharose and CM-Sepharose chromatography. The molecular weight of the purified APase was estimated to be 51 kDa judged by SDS-PAGE. The N-terminal 15 amino acid sequence of the purified APase was determined; (NH_2)-KEFPSTDIPLESEWF-. This sequence is very similar to the partial N-terminal amino acid sequence of the soluble APase from maize roots. The optimal pH of this enzyme was 5.5 and its optimal temperature was 65℃. The 51 kDa APase was severely inhibited by zinc ion and other general inhibitors like molybdate, pyrophosphate, fluoride and vanadate. Inorganic phosphate was also an inhibitor to this enzyme and its IC_{50} was 10.2mM. DTT and *N*-ethylmaleimide did not significantly affect the enzyme activity. The apparent *Km* for *p*-nitrophenyl phosphate(*p*NPP) was estimated to be 1.54mM and its *Vmax* was 5.67 μmole *p*-nitrophenol/min · mg. The purified APase displayed the highest affinity for *p*NPP and it showed a broad substrate specificity for ATP, ADP, AMP, phospho*enol*pyruvate, α-naphthyl phosphate and pyrophosphate. Based on the biochemical characterization of 51 kDa APase, it is very similar to 52 kDa of APase from maize roots. These results should help a better understanding of cellular functions of 51 kDa APase present in the coleoptiles.

E226 The changes in ethylene product during tomato (*Lycopersicon esculentum* Mill.) leaf senescence

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A δ-aminolevulinic acid dehydratase(ALAD: EC. 4.2.1.24) which is the key enzyme of tetraphyrrole biosynthesis has been purified from Jerusalem artichoke (*Helianthus tuberosus* L.). Purification was carried out by ammonium sulfate precipitation, molecular sieving, and ion exchange chromatography on Prep-Bio-gel, Toyo pearl HW-55 and DEAE Toyo pearl LP chromatography system. The enzyme was purified about 110-fold and showed a final specific activity of 6.32 nKat/g protein at a total recovery of 6%. The molecular mass of the native enzyme was determined by gel filtration to be approximately 320 kDa. SDS-PAGE showed two bands of 105 kDa and 56 kDa. This suggested the holozyme might be a dimeric protein composed with two of each subunit. The *Km* value of the ALAD was to be 0.75 mM at a *Vmax* of 250 μmol/min. Mg^{2+} was determined to be the metal cofactor of the enzyme. It can, to a certain extent, be substitute by other divalent cations except Zn^{2+}. ALAD from Jerusalem artichoke was completely inhibited by low concentration of $ZnCl_2$.