5 Biochemical characterization of 52kD acid phosphatase from maize roots

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The soluble acid phosphatase (APase, EC 3.1.3.2) from maize (*Zea mays* L.) roots was purified to near homogeneity by polyethylene glycol fractionation, DEAE-Sepharose, Con A-Sepharose and CM-Sepharose chromatography. The molecular weight of the purified APase was estimated to be 52 kDa judged by SDS-PAGE. The N-terminal 12 amino acid sequence of the purified APase was determined; (NH_2)-EFPSTDIPLESE-. This sequence had 58% identity with the partial N-terminal amino acid sequence of the cytosolic APase from pea plumules. The optimal pH of this enzyme was 5.5 and its optimal temperature was 60℃. The 52 kDa APase was severely inhibited by zinc ion and other general inhibitors like molybdate, fluoride and vanadate. Inorganic phosphate was also an inhibitor to this enzyme and its IC_{50}> was 12.2mM. But Mg^{2+}, Ca^{2+}, Mn^{2+} and EDTA did not affect the enzyme activity. DTT and *N*-ethylmaleimide did not also significantly affect the enzyme activity. In addition, okadaic acid had no effect on the 52 kDa APase activity. The apparent *Km* for *p*-nitrophenyl phosphate (*p*NPP) was estimated to be 0.7mM and its *Vmax* was 80 μmole *p*-nitrophenol/min · mg. The purified APase displayed the highest affinity for pNPP and it showed a broad substrate specificity for ATP, ADP, AMP, phosphoenolpyruvate(PEP), α-naphthyl phosphate and pyrophosphate. The root sections incubated in the Pi-deficient condition showed a stronger APase activity than those in the Pi-sufficient condition did, when the activity was measured *in situ* on non-denaturing gel. It was also shown that the phosphate deficiency increased the specific activity of APase to two folds, based on the spectrometric assay. These results should help a better understanding of APase function *in vivo*.

Keywords: maize roots, acid phosphatase, phosphate deficiency