The Molecular Characterization and Cloning of Mannitol-1-Phosphate Dehydrogenase that Contributes to the Salt Tolerance in Plants

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Summary

Mannitol, as a compatible solute, acts in plants as osmoregulator and as storage substance in bacteria and fungi. In the former study, the authors suggested the importance of mannitol-1-phosphate dehydrogenase (M1PDH) for the efficient regulation of the biosynthesis of mannitol in the red alga *Caloglossa continua* (Iwamoto *et al.*, *Mar. Biotechnol.*, 3: 493-500, 2001). Hence, in this study, we attempted to purify M1PDH to characterize biochemical properties of the enzyme and to obtain molecular informations much as the cDNA sequences of M1PDH in the red alga *C. continua.*

The enzyme M1PDH was purified by the combination of aqueous two-phase partitioning method with polyethylene glycol-ammonium sulfate, gel filtration by Sephadex G-25, affinity chromatography by Reactive Red 120 agarose, anion chromatography by BioAssist Q, and gel filtration by Sephacryl S-100. The enzyme was finally purified 50,957-fold, and its specific activity was shown to be 228 $\mu mol \cdot min^{-1}$ mg $protein^{-1}$. Since gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis gave close values of apparent molecular weights of 53,600 and 52,100, respectively, the enzyme was shown to be a monomer. The substrate specificity of the M1PDH was very high enough to metabolize only F6P and NADH for reduction, and M1P and NAD for oxidation. The enzyme was inhibited by *N*-ethylmaleimide, and *p*-hydroxymercuribenzoic acid (*p*CMB) and the inhibition by *p*CMB was rescued by the addition of 2-mercaptoethanol, indicatingthat the enzyme is a SH-enzyme. Zn^{2+} strongly inactivated, however Mg^{2+}, Mn^{2+}, Ca^{2+} did not affect the activity. The pH optimum for reduction was pH 7.0 in the presence of 200 mM NaCl, and it was shifted to under 5.0 in the absence of NaCl. Moreover, the kinetic analysis of M1PDH revealed that the affinity of the enzyme was greatly affected by NaCl. These findings indicated that the M1PDH activity was controlled by F6P concentration and also influenced NaCl concentration. Hence, it could be said that M1PDH is the key enzyme for regulation of mannitol biosynthesis *in vivo.*