The Molecular Cloning of Enzymes Catalyzing the Biosynthesis of Mannitol that Contribute to the Salt Tolerance in Plants

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Summary

Recently some attempts have been made to produce the salt tolerant transgenic plant by introducing the mechanism of mannitol biosynthesis. Though the transgenic plant accumulated the mannitol and showed the salt tolerance to some extent, the tolerance of the plant was not strong. There is a need to increase the salt tolerance of plants. The authors proposed, one of the solutions, to transfer the gene of mannitol-1-phosphatase (M1Pase) to be added to the gene of mannitol-1-phosphate dehydrogenase (M1PDH) that had only been transferred in former studies. M1Pase plays an important role in plant mannitol metabolism, because it catalyzes the final step of mannitol biosynthesis, M1P to mannitol. However, there is no information on molecular properties of M1Pase from plant. In this study, the authors intended to purify the M1Pase and to have some molecular information required to determine the cDNA sequences of M1Pase from the red alga *Caloglossa continua* (Okamura) King et Puttock.

The enzyme was purified by the combination of aqueous two-phase partitioning method with polyethylene glycol-ammonium sulfate, ammonium sulfate precipitation, and chromatographies on phenyl-Toyopearl, butyl-Toyopearl, Sephacryl S-100, Mono-Q, and Superdex 200 HR. The enzyme was shown to be a monomer, since gel-filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis gave close values of apparent molecular weights of 28,500 and 30,200, respectively. The protein exhibited an isoelectric point of 4.8. The substrate specificity for mannitol-1-phosphate (M1P) was very high, and that for K_m(M1P) was 0.41 mM. The catalytic activity was optimal at pH 7.4. The enzyme was activated by Mg^{2+}, but was strongly inhibited by Ca^{2+}, NaF, *N*-ethylmaleimide, and *p*-hydroxymercuribenzoic acid. Seawater levels of NaCl and physiological levels of mannitol also inhibited the activity by 50% or more. Changes in the concentrations of those ions and metabolites may regulate the biosynthesis of mannitol as an osmoregulant *in vivo*.

These findings were revealed for the advancement in transferring the gene of M1Pase, because the enzyme will be active in the cytosol that is with neutral pH condition, and the accumulation of mannitol in the cytosol can be controlled. The enzyme would not influence the metabolic system since it is highly specific to M1P and feedback regulation of the enzyme activity by the mannitol maintains its concentration at a proper level.