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Orchardgrass Hsp70 stabilizes heat-denatured protein and associates with chaperone complex

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Objectives

We demonstrate that orchardgrass Hsp70 assists the refolding of denatured proteins and it associates with different chaperone complex.

Materials and Methods

Orchardgrass (*Dactylis glomerata* L.) Hsp70 was isolated from a cDNA library and cloned into pET for protein expression, pYES2 for yeast transformation and pBD or pAD-GAL4 for yeast two-hybrid. Two-week-old orchardgrass seedlings were treated various stresses and used for northern blot analysis. Thermotolerance was carried out with W303 wild-type yeast strain harbouring Hsp70::pYES2 or pYES2 vector. Chaperone activity was measured by activation of Hsp70 to prevent thermally aggregating MDH (malate dehydrogenase). ATPase assay was performed as radioactivity of hydrolyzed [α-^{32}P]ATP on PEI cellulose plate. Protein-protein interaction was confirmed by yeast two-hybrid protocols (Stratagene Co.).

Results and Discussion

Hsp70 (Heat shock protein 70) functions as a molecular chaperone in a variety of cellular processes to cope with a variety of unfavorable environmental conditions. Recent studies have elucidated that Hsp70 chaperone functions under stress conditions and in the protein metabolism, such as folding, disaggregation and renaturation of non-native proteins. It also mediates the assembly and disassembly of multimeric protein complexes. In this study, we report the characteristics of orchardgrass Hsp70 and its chaperone function related to chaperone complexes. Expression of *Hsp70* was highly enhanced by heat, hydrogen peroxide and ethanol treatment. Orchardgrass Hsp70 contains conserved ATPase domain in N terminal and TPR (Tetratricopeptide repeat) domain in C terminal, respectively. It was able to bind non-native protein and strongly inhibited the thermal induced aggregation of malate dehydrogenase, *in vitro*. We also confirmed that the overexpression of Hsp70 correlated positively with the acquisition of thermotolerance in wild type yeast cells. These results show that Hsp70 has important role in cell survival under stress conditions. Recently, a few studies have been reported that one of small Hsp cooperates with mammalian Hsp70 system to reactivate a heat-denatured protein. To investigate the role of small Hsps in Hsp70 system in plant, we isolated Arabidopsis Hsp70 and five kinds of differently localized small Hsps (cytosolic I, cytosolic II, chroloplastic, mitochondrial and endosperm reticulum) by RT-PCR. AtHsp70 revealed to interact with all small Hsps. Elucidating the role of Hsp70 among Hsp90 chaperone machinery (Hsp90, Hsp70, Sti/Hop, p40 and p23) is in progress.

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