

## Wheat dehydrin DHN-5, their K-Segments, exerts a heat-protective effect and protection role during some enzymatic activities

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Group-2 Late Embryogenesis Abundant (LEA) proteins, also known as dehydrins, are claimed to stabilize macromolecules against damage caused by freezing, dehydration, ionic or osmotic stresses. However, their precise function remains unknown. We investigated the effect of wheat dehydrin (DHN-5) protein on the activity and thermostability of two distinct enzymes,  $\beta$ -glucosidase (bglG) and glucose oxidase/peroxidase (GOD/POD) *in vitro*. The purified DHN-5 protein had the capacity to preserve and stabilize the activity of bglG subjected to heat treatment. In addition, DHN-5 stabilized oxidizing enzymes, as it improved reliability in measuring glucose concentrations with a glucose oxidase/peroxidase (GOD/POD) kit while the temperature increased from 37 to 70°C. All together the data presented provide evidence that DHN-5 is a dehydrin able to preserve enzyme activities *in vitro* from adverse effects induced by heating. In order to understand the molecular mechanism by which DHN-5 exerts its protective function, we performed an approach to dissect the functional domains of DHN-5 responsible for this feature. In two distinct enzymatic assays, we found that the truncated forms of DHN-5 containing only one K- or two K-segments are able to protect albeit to less extent than the wild type protein, lactate dehydrogenase and  $\beta$ -glucosidase against damage induced by various stresses *in vitro*. However, the YS- and  $\phi$ -segments alone have no protective effects on these enzymes. Therefore, our study provides the evidence that the protective function of DHN-5 seems to be directly linked to its K-segments which through their amphipathic  $\alpha$ -helical structure, may act to prevent protein aggregation.

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