

Purification, immunolocalization, cryoprotective, and antifreeze activity of PCA60: A dehydrin from peach (*Prunus persica*)

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Dehydrins are glycine-rich, hydrophilic, heat-stable proteins and are generally induced in response to a wide array of environmental stresses. In previous research (Artlip et al. 1997, *Plant Molecular Biology* 33: 61–70), a full-length dehydrin gene, *ppdh1*, was isolated from peach, and its expression was associated with qualitative and quantitative differences in cold hardiness in sibling genotypes of evergreen and deciduous peach. Similar results were obtained for levels of the corresponding 60 kDa peach dehydrin protein (PCA60). The objective of the present study was to purify the PCA60, test the purified protein for cryoprotective and/or antifreeze activity, and to determine the cellular localization of PCA60 using immunomicroscopy. PCA60 was extracted from winter bark tissues of peach (*Prunus persica* [L.] Batsch) and purified in a two-step process. Separation was based on free-solution

isoelectric focusing followed by size exclusion. Purified PCA60, as well as crude protein extract, preserved the *in vitro* enzymatic activity of lactate dehydrogenase after several freeze-thaw cycles in liquid nitrogen. PCA also exhibited distinct antifreeze activity as evidenced by ice crystal morphology and thermal hysteresis. This is the first time antifreeze activity has been demonstrated for dehydrins. Immunomicroscopy, utilizing an affinity-purified, polyclonal antibody developed against a synthetic peptide of the lysine-rich consensus portion of dehydrins, indicated that PCA60 was freely distributed in the cytoplasm, plastids, and nucleus of bark cells and xylem parenchyma cells. Although the functional role of dehydrins remains speculative, the data support the hypothesis that it plays a role in preventing denaturation of proteins exposed to dehydrative stresses.

Introduction

The dehydrin family of proteins is induced by environmental stresses that result in cellular dehydration (Close 1997). They are glycine-rich proteins, deficient in tryptophan and cysteine, and remain soluble at temperatures approaching 100°C. In addition, dehydrins are characterized by a lysine-rich amino acid sequence present at the carboxy terminus and often repeated throughout the polypeptide. The polypeptide is believed to form an amphipathic helix and might interact with lipids in a manner similar to the interaction of class A amphipathic α -helices of apolipoproteins with lipids, or with exposed hydrophobic patches of partially denatured proteins (Close 1997) in a manner similar to chaperones (Mayhew and Hartl 1996). Thus, dehydrins may

serve a protective function during environmental stress (Dure 1993, Close 1997).

While much has been learned about dehydrins in herbaceous plants, information on dehydrins in woody plants is limited. Seasonal expression of dehydrins has been noted in several species (Wisniewski et al. 1996) including peach (*Prunus persica*) (Arora and Wisniewski 1994) and blueberry (*Vaccinium*, sec. *Cyanococcus*) (Muthalif and Rowland 1994). Chilling and desiccation have also been shown to induce dehydrin gene expression in citrus (*Poncirus trifoliata* and *Citrus grandis*) leaves (Cai et al. 1995). Regarding woody plants, dehydrins have been most extensively characterized in peach, where association of dehydrins with differ-

Abbreviations – AFP: antifreeze protein; BSA: bovine serum albumin; LDH: lactate dehydrogenase; PBS: phosphate buffered saline.