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Molecular mechanism of dehydrin in response to environmental stress in plant*

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Abstract Dehydrins, known as the D-11 subgroup of late embryogenesis abundant (LEA) protein, are an immunologically distinct family of proteins, which typically accumulate in desiccation-tolerant seed embryo or in vegetative tissues in response to various environmental stresses such as drought, salinity and freezing. The existence of conservative sequences designated as K, S, and Y segments is a structural feature of dehydrins, and the K segment found in all dehydrins represents a highly conserved 15 amino acid motif (EKKGIMDKIKEKLPG) and forms an amphiphilic α-helix. According to the arrangement of these domains and clustering analysis, dehydrins are subdivided into 5 subtypes: YnSK, Kn, KnS, SKn and YnK. Different types of dehydrins are induced by different environmental stress in plants. Study results showed that dehydrins might play important protective roles under abiotic stress via a number of different mechanisms, including improving or protecting enzyme activities by the cryoprotective activity in responding to freeze/thaw or dehydration; stabilizing vesicles or other endomembrane structures by function as the membrane stabilizer during freeze induced dehydration, and preventing the membrane system from the oxidative damage induced by reactive oxygen radicals as the radical scavenger. Here, the gene expression and molecular mechanisms of dehydrin in response to stress in plants are discussed.

Keywords: **dehydrin, stress, mechanism.**

Environmental stresses, such as drought, cold and high salinity, are major limiting factors for plant growth and development. Plants have evolved a wide variety of molecular responses to enable them to survive severe abiotic stresses at cellular, tissue and whole plant levels. These responses are not only dependent upon the severity and duration of stress, but also on the developmental stage and morphological/anatomical properties of the plants[1]. Many genes respond experimentally to abiotic stresses, such as transcription factors, protein kinases, enzymes for the biosynthesis of osmoprotectants, radical-scavenging enzymes, water channels, late embryogenesis abundant (LEA) proteins and so on[2]. Although a large number of stress-induced genes have been identified in a wide range of plant species, the functions of other genes, such as those for LEA proteins, remain far from being completely understood.

Many proteins induced by dehydration are referred to as LEA proteins[3]. Dehydrin (DHN), also known as the D-11 subgroup of LEA protein, is the most studied among drought induced water soluble proteins. The first published sequence of dehydrin was the RAB21 protein of rice[4]. Today over 100 dehydrin genes have been characterized from both angiosperms and gymnosperms and distributed in a wide range of organisms, algae, yeast and cyanobacteria[5]. Dehydrins were first identified as being expressed during the process of seed maturation and dessication, subsequently were found to also be expressed in vegetative tissues in response to abscisic acid (ABA), drought, salinity, low temperature and heavy metal[6]. Numerous studies reported a correlation between the accumulation of dehydrin transcripts or proteins and the tolerance of abiotic stress[7—11]. The *PpDhnA* knockout mutant of moss showed severely impaired in its capacity to resume growth after salt and osmotic-stress treatments, providing direct genetic evidence in any plant species for a dehydrin exerting a protective role during cellular dehydration allowing recovery when returned to optimal growth conditions[12]. Furthermore, in *Arabidopsis thaliana*, ERD14 was present in the vascular tissue and bordering parenchymal cells, LTI29 and ERD14 accumulated in the root tip, and RAB18 was localized to the stomatal guard cells, preferentially in the nuclei, indicating dehydrin was tissue- and cell type-specific in unstressed plants, and was involved in the

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** To whom correspondence should be addressed. E-mail: zhangyuxiu@cumtb.edu.cn metabolic processes of stomatal movement and water transportation[13]. However, these dehydrins exhibited clear differences in their accumulation patterns in response to stess. ERD14 protein level was up-regulated by ABA, salinity and low temperature; LTI29 mainly accumulated in response to low temperature, but was also found in ABA- and salt-treated plants. LTI30 and COR47 were not detected in unstressed plants, and accumulated primarily in response to low temperature, whereas RAB18 was only found in ABA-treated plants[14]. The differences in stress specificity and spatial distribution of dehydrins in one plant suggested a functional specialization for the members of this protein family.

Recently, results from transgenic plant, enzyme activity and conformation analysis *in vitro* revealed dehydrin played important roles in abiotic stress, such as membrane stabilizer, cryoprotective activities for freeze-sensitive enzymes, metal ion transport protein, and radical scavenger. Here, we discuss the structure, function and molecular mechanism of dehydrin under various environmental stress.

1 Structure and property of dehydrin

Dehydrins are generally highly hydrophilic, having a wide range of molecular masses from 9 to 200 kD, enrich with glycine, in addition to lysine, lack Trp and most often Cys residues, and contain a high proportion of charged and polar amino acids and a low proportion of nonpolar, hydrophobic residues[5]. This polar/charged amino acid compositional bias explains the high temperature solubility of dehydrins[6]. For example, analysis of the amino acid composition of a 35 kD dehydrin isolated from seedlings of cowpea revealed that it was characterized by a high content of Gly, Thr, Ser, Asp and Glu[15], indicating the highly hydrophilic nature of this protein. *Arabidopsis* LTI30 with molecular mass of 21 kD, the Gly, His, Lys, and Thr represented 56% of the total amino acids, whereas Cys, Trp, Arg and Val were not found[16].

Dehydrins are characterized by the three conservative motifs as the Y, S and K segments[5]. The Y-segment (T/VDEYGNP), when present, occurs in 1—3 tandem copies near the N-terminus. This sequence shares significant homology with the nucleotide binding site of plant and bacterial chaperones[5,6], however, binding of dehydrin Y segments with nucleotides remains to be well documented. The Ser tract repeats (S segment) that can undergo phosphorylation and are thought to promote dehydrin interaction with specific signal peptides followed by their translocation into the nucleus[17]. The K segment is a highly conservative Lys rich motif consisting of 15 amino acid residues and it has two types: the angiosperm-type (EKKGIMDKIKEKLPG) and the gymnosperm-type ((Q/E)K(P/A)G(M/L)LDKIK(A/Q)(K/M)(I/L)PG)[18]. K segments are usually located in the C terminus and maybe repeated 1 to 12 times. Computer aided analysis of amino acid sequences of K segments demonstrated the possibility of formation of class A2 amphipathic α-helix[19]. Circular dichroism (CD) data also supported formation of secondary α-helical structure by the K segments in maize[20], soybean[21] and cowpea[15,20]. The class A2 amphipathic α-helix forms two surfaces of different characteristics, where one side is formed by negatively charged amino acids and the opposite by hydrophobic amino acids and the positively charged residues form the interphase between the two surfaces[6]. In fact, the class A2 α-helix occurred in apolipoproteins which facilitate the transport of water-insoluble lipids in plasma through their lipid-associating characteristics, and the amphipathic helices are responsible for the lipid-associating properties[22]. Furthermore, with a number of similarities between dehydrins and apolipoproteins including high temperature solubility, a lack of Cys and Trp, and anomalous migration in SDS-PAGE, it seemed reasonable to hypothesize that the presence of amphipathic helices enables dehydrin to act as an interface between with hydrophobic surfaces of membrane phospholipids and the cytosol, or interact with exposed hydrophobic surfaces of partially denatured proteins to prevent protein-protein aggregation under conditions of low protoplasmic water activity arising from dehydration or freezing stress conditions[5]. Besides the Y, S and K conservative segments, dehydrins also contain less conservative regions, named ϕ segments, which enriched with Gly and polar amino acid residues (especially Thr)[5,6]. Thus, the size of dehydrin molecules, varied from 82 to 575 amino acid residues, is determined by combination and number of K, S, Y and ϕ segments[5,6].

Dehydrin does not fold into a well-defined three-dimensional (3D) structure in terms of the canonical secondary structural elements under native or physiological conditions. CD analyses of a purified dehydrin G50 from maize kernels showed approximately 75%

random conformation[20]. *Arabidopsis* ERD10 was practically fully disordered[23]. Soybean rGmDHN1 has a very low intrinsic tendency to adopt α-helical conformations in solution[21]. Nuclear magnetic resonance (NMR) spectral analysis suggested that the purified recombinant DSP16 from the drought-tolerant resurrection plant (*Craterostigma plantagineum*) formed a highly mobile unordered conformation in equilibrium with preferentially extended substructures having different conformational states[24]. The disordered structure of dehydrin conferred specific advantages, such as specificity without excessive binding strength[23] and the thermostability and maintained their integrity in aqueous solutions at up to 100℃[6]. Proton NMR intensity and differential scanning calorimetry (DSC) measurements on ERD10 reflected a much larger binding capacity for water of ERD10 than a globular control protein bovine serum albumin (BSA)[23]. Probably the mechanism of protection from the loss of water is underlined by their highly hydrophilic and intrinsically unstructured nature. However, dehydrin is structured in association with lipid vesicles or in the structure-making additives (such as trifluoroethanol) and SDS. The CD spectrum of free maize DHN1 showed its conformation was a random coil, whereas incubation of DHN1 with phosphatidic acid (PA)-derived small unilamellar vesicles (SUV) caused a significant change of structure, with 9% α-helix[25]. UV absorption and CD showed that the purified rGmDHN1 existed in equilibrium between two extended conformational states at different temperatures: unordered and poly (L-proline)-type Ⅱ structure[21]. Because of unordered conformation, such as random coil, was characterized by the formation of a layer cohesive to other structures and by water-binding activity, dehydrins might undergo function-related conformation changes at the water/membrane interface, related to the stabilization of vesicles or endomembrane structures under stress conditions.

2 The expression of different sub-type dehydrins in response to stress

Dehydrins were categorized into 5 sub-types based on number and position of the three conserved motifs, such as YnSKn, Kn, KnS, SKn and YnKn types[5]. From 37 dehydrin sequences deposited in GenBank, we performed cluster analysis (Fig. 1) by ClustalW and confirmed the 5 sub-types of dehydrins. It is evident that the different class of dehydrin was not identical in responding to various stresses. For instance, the YnSKn type generally responds to drought and ABA; Kn and KnS types respond to chilling and freezing; the SKn type mainly responds to low temperature, and also to other stresses, such as salinity, drought, wounding and heavy metals; while YnKn type showed no preference to any of the abiotic stress up to now.

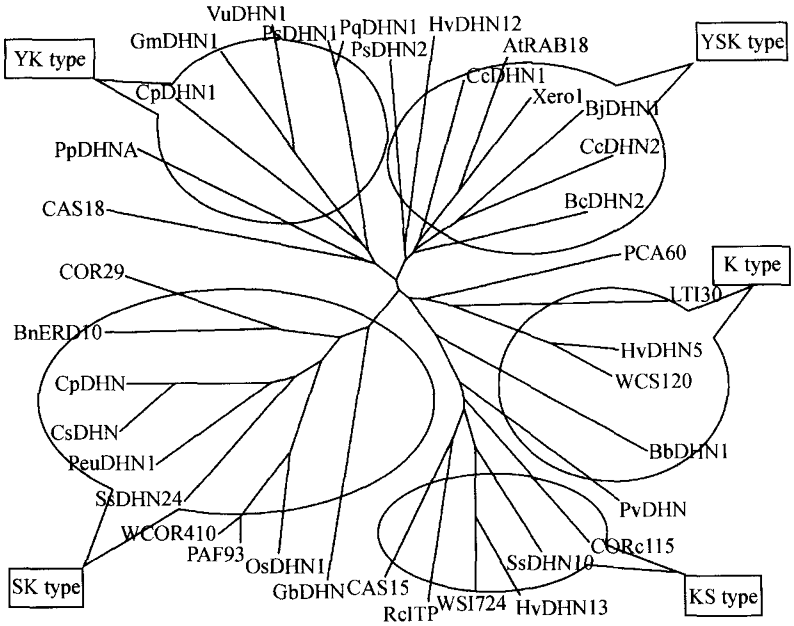

Fig. 1. The unrooted tree based on deduced amino acid sequences of dehydrins constructed using the ClustalW and TreeView program.

2.1 YnSKn dehydrins

This is the most abundant class of dehydrin that contains one to three Y segments, one S segment and two K segments. Most of dehydrins from sunflower and barley were described as "Y_3SK_2" and "Y_2SK_2", respectively[26,27]. *HaDhn*, isolated from different species of sunflower, shares the common structure Y_2SK_2 and its transcript accumulation has been found being related to drought tolerance[26]. Barley dehydrins, such as DHN1, DHN2, DHN3, DHN4, DHN6 and DHN9, sharing the common structure YSK_2, are basic or neutral proteins, which are upregulated by both dehydration and ABA treatment in seedlings as well as during the late stage of embryo development, but not by cold treatment[28]. *Coffea CcDh2*, encoding a Y_3SK_2 dehydrin, was expressed during the late stages of grain development. Promoter sequence analysis of *CcDh2* showed that there were several putative regulatory elements implicated in the control of both seed- and osmotic stress-specific gene expression[29]. Northern blot and promoter activity analysis by transgenic tobacco plant showed the *Boea crassifolia BcDh2* (YSK_2) was highly expressed when the plants were treated with drought, salinity, ABA and moderated heat shock, while slightly induced by low temperature[30]. The *Brassica* BjDHN1 (Y_3SK_2) was mainly accumulated at the late stages of developing siliques, and induced in germinating seeds by low temperature[31]. These suggested that the YnSKn-type dehydrins may be mainly responsive to drought and ABA.

2.2 Kn dehydrins

Dehydrins without S and Y motifs are a rather rare class, most of which are acidic or neutral proteins containing two to nine K segments, and are strongly induced by low temperature. For example, pistachio (*Pistacia vera Pvdhn*) encodes a K_5-type dehydrin its mRNA is most abundant in winter, suggesting that Kn-type dehydrin is cold-induced[32]. Similarly, the accumulation of *Arabidopsis* LTI30 (K_6) was only induced by low temperature[33]. Expression of barley *Dhn5* encoding a K_9 dehydrin was induced by low temperature and to a lesser extent by drought and ABA[34]. Soft wheat WCS120 protein family was homologous to barley DHN5, synthesis of this protein was specifically induced under low temperature and the level of protein accumulation positively correlates with chilling tolerance of wheat[35]. In blueberry, several Kn-type dehydrins were also characterized, both the 60 and 65 kD dehydrins (K_5) appeared to be strongly induced by cold and slightly by drought in floral buds, and the K_2-type 14 kD dehydrin was also strongly induced by cold and to a lesser extent by drought stress in flower buds with its mRNA induced to higher levels in the cold hardy and drought tolerant genotype[36]. In addition, wheat *Wdhn13* encoding a K_2 dehydrin was predominantly responsive to low temperature, and also to dehydration stress to a less extent[37].

2.3 KnS dehydrins

In contrast to other classes of dehydrins, the K segment of KnS dehydrins begins with the motif (H/Q)KEG[38] (in others a similar site contains EKKG), this is the characteristic feature of these proteins. Barley DHN13 contains one K segment (HKEGMMEKIKDKISG) at position 46—61 and one S segment (SSSSSDSD) at the carboxy terminus. Semi-quantitative RT-PCR data indicated that *Dhn13* was constitutively expressed in seedling tissues and embryos of developing seeds. Microarray data were consistent with these results and showed a considerable increase of *Dhn13* transcripts when plants were subjected to chilling and freezing[39]. In wild potato, DHN10 was a member of a subclass of KnS-type dehydrins, the K- and S-segments only occur in single copies when the S-segment is at a C-terminal position. DHN10 abundance was increased only in mature expanded leaves when two *Solanum* species subjected to low temperature or to drought[40,41]. The increased abundance in mature leaves under stress may compensate for a decrease in metal transport due to a lower transpiration flux. Citrus CuCOR19 (K_3S) is highly hydrophilic and contains three repeating elements including Lys-rich motifs, its expression in leaves increased by cold stress, overexpression of *CuCOR19* in transgenic tobacco plant enhanced cold tolerance[42,43].

2.4 SKn dehydrins

Proteins of this class contain one S segment and two or three K segments. The high percentage of acidic and basic amino acids reveals this subclass proteins are acidic dehydrins, which are preferentially induced by low temperature, and tend to be responsive to other abiotic stresses, such as salinity, drought heavy metal, wounding and jasmonate (JA). Wheat WCOR410 (SK_3) is accumulated in the vascular transition area where freezing induced dehydration was severe[44]. DHN24 (SK_3) originated from a cold-acclimated species *Solanum sogarandinum* was essentially linked to low temperature tolerance in cold-sensitive species such as cucumber and could be used to improve chilling/freezing tolerance through a transgenic approach[45]. The transcripts of rice *OsDhn1* (SK_3) induced by cold stress were detected in the roots of seedling and the epidermis of palea and lemma[46]. The *Arabidopsis* acidic SK_3-type dehydrin LTI29 was mainly accumulated in response to low temperature in most cells, while the ERD14 (SK_2) was up-regulated by ABA, salinity and low temperature[13]. Birch *BpuDhn2* encoded an acidic SK_2-type of DHN, its mRNA level was highest during the coldest winter months in field-grown trees, whereas under controlled conditions the expression of *BpuDhn2* was mainly controlled by low temperature while photoperiod had less effect on its expression[47]. The *PgDhn1* encoding a SK_4 dehydrin in spruce was increased in needle tissue under the stress of low temperature, drought, salinity, wound and treatment with JA and methyl jasmonate (MeJa)[48]. The expression of *CpDhn* (SK_2) in *Citrus paradisi* was specifically induced in the fruit peel tissue by exposure to heat followed by chilling[18]. *PvSR3* encoding an SK_2 dehydrin in french bean was up-regulated by heavy metals[49]. RT-PCR analysis revealed that the GbDHN (SK) from the living fossil plant ginkgo was accumulated by exogenous ABA, salt and drought[50]. Similarly, the transcripts of *BnDhn* in *Brassica napus* encoding a SK_2 dehydrin were increased upon cold, salt and ABA stresses in young leaves[51]. The expression of *Cbcor29* (SK_3) from wild chickpea (*Capsella bursa-pastoris*) was induced when exposed to drought, low-temperature, NaCl or exogenous ABA, respectively[52].

2.5 YnKn dehydrins

This class of dehydrins usually contains two Y segments and one or two K segments. This subgroup is up-regulated by stresses, however, there is still lack of evidence showing the preference to any of the abiotic stress. Birch *BpuDhn1* encoding an Y_3K_2-type of basic DHN was increased in buds during the autumn in field-grown trees, under controlled conditions BpuDhn1 increased in response to the combined effect of short daylength and low, non-freezing temperatures[47]. Cowpea 35 kD dehydrin (Y_2K) was found to be associated with seed chilling tolerance during germination[15]. Chickpea *cpdhn1* encodes a Y_2K-type dehydrin, and it can be induced not only during seed development, but also in leaves in response to stress, such as drought, chilling and salinity, in addition to treatment with ABA or MeJa, suggesting CpDHN1 might improve the tolerance of chickpea to a variety of environmental stresses[53]. The expression of soybean *Matl*, which encodes a Y_2K-type dehydrin, was dehydration dependent[54]. Birch *BpuDhn1* encodes a Y_3K_2-type of basic DHN, and it increased in buds during the autumn in field-grown trees, under controlled conditions BpuDhn1 increased in response to the combined effect of short daylength and low, non-freezing temperatures[47].

3 Molecular mechanisms of dehydrin in response to stress

3.1 Stabilization of membrane structure

The dehydration process may damage integrity of hydrate cover of macromolecules, this, in turn, may result in shortening of the distance between membrane bilayers, mixing their components and finally irreversibly changing the structure of biomembranes[44]. Dehydrin was proposed to function as the membrane stabilizer during freeze induced dehydration[5,6].

WCOR410 is a peripheral protein and accumulates in the vicinity of the plasma membrane of specific cell types. The high content of acidic, basic and hydroxylated amino acids was the basis for preventing lipid demixing that occurs during dehydration, and the highly charged nature of WCOR410 also suggested that it could retain or directly replace water for the "solvation" of the membrane through polar interactions[55], indicating that WCOR410 might play a role in preventing the destabilization of the plasma membrane and their abundant association with the plasma membrane of these cells could cause a steric hindrance to the interaction between membrane bilayers[44]. *Arabidopsis* COR15a increased the lamellar-to-hexagonal II phase transition temperature of dioleoylphosphatidylethanolamine and promoted formation of the lamellar phase in a lipid mixture composed of the major lipid species that comprise the chloroplast envelope, suggesting that COR15a, located in the chloroplast stroma, defers freeze-induced formation of the hexagonal II phase to lower temperatures (lower hydrations) by altering the intrinsic curvature of the inner membrane of the chloroplast envelope[56].

Class A2 amphipathic α-helices were thought to stabilize the membrane, and protect it from fusion and lysis by decreasing the monolayer negative curvature strain[56]. Maize DHN1 could bind to lipid vesicles containing PA and that association of DHN1 with vesicles resulted in an apparent increase of an α-helicity of the protein[25]. The presence of amphipathic helices in DHN1 suggested that dehydrin might act as an interface between hydrophobic surfaces of membrane phospholipids and the cytosol in plant cells. Therefore, maize DHN1 might stabilize membranes either by reducing the negative curvature strain of PA-enriched monolayers, possibly to inhibit transition to the hexagonal II phase, or by altering the membrane interfacial charge density to decrease the facilitated fusion of negatively charged vesicles.

3.2 The cryoprotective activity

Dehydrins are induced in plants exposed to water stress or low temperature, and have been associated with cold acclimation. Accumulation of both DHN5 and DHN8 in barley is in association with some induction of freezing tolerance[28]. The presence of the 35 kD dehydrin in cowpea is associated with chilling tolerance during seedling emergence and the allelic variation at the dehydrin gene locus co-segregates with chilling tolerance[15]. Dehydrin family members in *Citrus*, such as Cor11, Cor19, Crcor15 and CuCOR19, were involved in the acquisition of low temperature tolerance[18,43]. Birch BpuDHN1 and BpuDHN2 participated in the sensitive environmental regulation of the overwintering process[47]. In transgenic *Arabidopsis* plants, overexpressing double constructs for expression of RAB18 (Y_2SK_2) and COR47 (SK_3) or LTI29 (SK_3) and LTI30 (K_6) driven by the cauliflower mosaic virus (CMV) 35S promoter exhibited lower LT50 values (low temperature value) and improved survival when exposed to freezing stress compared to the control plants[14]. The precise role of DHNs in cold resistance remains unclear. It has been proposed, however, that they act as cryoprotectants by stabilizing macromolecules or cellular structures under a reduced hydration state caused by freezing[5,6].

The accumulation of peach PCA60 distributed in the cytoplasm, plastids, and nucleus of bark cells and xylem parenchyma cells was correlated with the level of cold hardiness. Using a nanoliter osmometer and phase-contrast photomicroscope, it was found that the formation of ice crystals shaped as hexagonal bipyramids in solutions of PCA60, indicating a fairly high level of antifreeze activity due to its hydrophilicity and concomitant ability to bind water through binding to ice surfaces and inhibiting the ability of water molecules to join ice lattices, as was generally proposed for antifreeze proteins (AFPs)[57]. A 24 kD dehydrin isolated from purified starch granules (SGs) in the apex of birch was produced during cold acclimation by immunolocalization and Western blots, moreover, the hydrolyzation activity of α-amylase was enhanced with the accumulation of 24 kD dehydrin[58], indicating dehydrin could improve hydrolase function of α-amylase during cold acclimation. In cold-induce dehydrating cells, the hydrophilic portions of dehydrins could cluster water molecules around enzymes, providing water as substrate and allowing the intramolecular motions necessary during enzyme/substrate association and subsequent enzyme action.

P-80 is a cold-induced 80 kD dehydrin in barley, and has the same apparent molecular mass as Dhn5[34]. P-80 is localized in the vicinity of vascular cylinders and in the epidermis of leaves and stems, both tissues have been reported to be sites of early ice nucleation during controlled freezing, suggesting this protein cryoprotects macromolecules and frost-sensitive structures[34]. The cryoprotective activity on freeze-thaw inactivation of enzymes was used as a criterion of the function of LEA proteins including dehydrins. Both P-80 and Dhn5 proteins were able to cryoprotect lactate dehydrogenase (LDH) against freeze/thaw inactivation *in vitro*, showing a similar shape dependence on concentration and almost the same protein dosage that renders 50% of cryoprotection (PD_{50})[34]. LDH inactivation assay showed that 20 $\mu g\ ml^{-1}$ of purified PCA60 maintained 50% of LDH activity after several freeze-thaw cycles in liquid nitrogen[57]. Similarly, the soybean 26/27 kD prevented LDH from freeze-thaw inactivation, the protein amount necessary to keep 50% of enzyme activity (CP_{50}) of the 27 kD dehydrin was slightly lower than 26 kD dehydrin. The reverse phase chromatogram of the trypsin digest implied disparity in the internal glycine-rich region between the dehydrins, and that the molecular diversity of dehydrin, presumably related to the hydrophilic glycine-rich region, might influence its function to protect proteins against abiotc stress[59].

The mechanism by which dehydrins cryoprotect LDH was unknown. The K segment in dehydrin, forming amphipathic a-helix, could interact with lipids or with exposed hydrophobic patches of partially denatured LDH preventing further inactivation. According to this, one expected a critical number of K repeats is necessary to exert cryoprotection or an special conformation of the whole protein may be obtained by tandem repeats of K segments[34]. However, this is not the case, because COR15a, a dehydrin with three K segments, is a seven times more effective cryoprotector of LDH than P-80 and Dhn5, and 50 times more effective than PCA60[34]. These three dehydrins contain nine K repeats. In addition, cryoprotection of LDH by a 20 amino acids synthetic peptide with one K segment is very low[34]. It is possible that a critical number of K repeats is necessary to exert cryoprotection or a special conformation of the whole protein may be obtained by tandem repeats of K segments[34]. In fact, most cold induced dehydrins, regardless of the species in which they were found, contain three or more K repeats within the polypeptide. Further studies of cryoprotection against freeze/thaw inactivation using dehydrins containing less than three K segments are necessary to elucidate this hypothesis.

3.3 The radical scavenger

Membrane structures are the most susceptible to stress. The membrane injury in cold stress was speculated to be the decrease in fluidity of membranes by peroxidation and lipid peroxidation. In plant, overexpression of citrus CuCOR19 in transgenic tobacco plant showed less electrolyte leakage and lower malondialdehyde (MDA) production than the control plant by chilling, indicating CuCOR19 enhanced cold tolerance by reducing lipid peroxidation and might directly scavenge radicals[43,60]. The hydroxyl radical and peroxyl radical were scavenged by CuCOR19, but hydrogen peroxide and superoxide were not. The scavenging activity for the hydroxyl radical and peroxyl radical of CuCOR19 was more potent than that of mannitol, and approximately equal to that of serum albumin, which is known as an antioxidative protein in mammals. CuCOR19 was degraded by the hydroxyl radical and peroxyl radical in a time- and dose-dependent manner[60]. Mannitol and thiourea inhibited the degradation. Analysis of the amino acid composition of CuCOR19 indicated that Gly, His, and Lys, which are major residues in many dehydrins, were targeted by the hydroxyl radical, suggesting that dehydrin was a radical scavenging protein, and may reduce oxidative damage induced by water stress in plants[60]. Furthermore, dehydrin was located mainly in the cytoplasm as well as in nucleus and mitochondria, DNA was also considered highly susceptible to radicals, thus, dehydrin might protect DNA from oxidative damage in nuclei and mitochondria under stress conditions except for membrane system[60].

3.4 The ion-binding activity

Metal ions strength increase is caused by membrane leakage during stress, while dehydrins possess ion binding property and prevent adverse increase in ionic strength, thus counter deleterious effects, such as protein denaturation. In fact, the interaction of dehydrins on immobilized metal ion affinity chromatography (IMAC) columns showed the ion-binding activity previously[16]. Lately, a KS-type dehydrin purified from castor bean, designated as ITP, was found to function as an iron transport protein that was thought to facilitate phloem-mediated long-distance transport of micronutrients[61]. The purified ITP preferentially binds Fe^{3+} over Fe^{2+} and complexes with Cu^{2+}, Zn^{2+}, and Mn^{2+} *in vitro*, whereas *in vivo* only complexed with iron[61]. Celery dehydrin-like protein VcaB45, located in the lumen of vacuole, was shown to bind 100-fold more calcium when phosphorylated and may act as a calcium buffer to help alleviate the elevated intracellular calcium concentrations caused by leakage across membranes that occurs during environmental stress or as a calcium-dependent chaperone[62]. In another case, *Arabidopsis* ERD14 accumulated in response to dehydration-related environment was capable of binding a variety of ions and the ion binding (calcium in particular) activity was also dependent upon the ERD14 phosphorylation *in vitro*[63]. Furthermore, the calcium-binding region of the acidic dehydrins may be upstream of the S-motif, on the amino terminal portion of these proteins[64].

Arabidopsis ERD14 was phosphorylated by extracts of cold-treated tissues *in vitro*[63], suggesting that the phosphorylation status of dehydrin might be modulated by cold-regulated kinases or phosphatases. Calcium binding properties of ERD14 purified from *Arabidopsis* extracts were comparable with phosphorylated *E. coli*-expressed ERD14[63]. Approximately 2 mol of phosphate were incorporated per mol of ERD14, indicating a minimum of two phosphorylation sites. Western blot analyses confirmed that Thr and Ser are possible phosphorylation sites on ERD14. Utilizing matrix assisted laser desorption ionization-time of flight/mass spectrometry, five phosphorylated peptides that were present in both *in vivo* and *in vitro* phosphorylated ERD14 were identified. The polyserine (S) domain is most likely the site of phosphorylation in ERD14 responsible for the activation of calcium binding[63].

More recently, the metal binding property of citrus dehydrin CuCOR15 was proved by IMAC[65]. Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} bound to citrus dehydrin, but Mg^{2+}, Ca^{2+}, and Mn^{2+} did not. The highest affinity was Cu^{2+}-dehydrin binding among the bound metals. Citrus dehydrin was able to bind up to 16 Cu^{2+} ions. The ion binding domain in CuCOR15 has been identified, and the core sequence for the binding is HKGEHHSGDHH. These results suggested that citrus dehydrin binds metals using a specific sequence containing His. The His-X3-His motif was characterized as a metal binding site in many metal-binding proteins, such as C2H2-type zinc finger transcription factors, zinc dependent metallopeptidases, histatins and synthetic peptides binding metals[65]. Most dehydrins contain a high proportion of His and possess the double His sequence and/or the His-X3-His Motif[65], suggesting that dehydrins may be metal-binding proteins. And metal ions, such as Fe^{2+} and Fe^{3+} or Cu^{+} and Cu^{2+}, can form hydroxyl radicals by reacting with hydrogen peroxide or superoxide anions. Since citrus dehydrin was a radical-scavenging protein[60] and able to bind metal ions, it might reduce metal toxicity in plant cells under water-stressed conditions.

In addtion, *Arabidopsis* ERD10 was the early responsive to dehydration, expressed in plants in certain very actively dividing tissues and ubiquitously under drying conditions[23]. Proton NMR intensity and DSC measurements overall provided direct evidence that the intrinsically unstructured ERD10 not only has a high hydration capacity but also binds a large amount of charged solute ions[23], indicating that function of dehydrin probably results form its simultaneous action of retaining water in the drying cells and preventing adverse increase in ionic strength[23].

4 Prospect

Over the past 20 years, studies on classification, expression, structure and function of dehydrin have made considerable progress. It is proposed that dehydrin may play roles in maintaining homeostasis under stress, such as membrane protection, chaperone action, water retention and ion sequestration. However, the protection mechanism of dehydrin in response to stress is far from understood. The structure and function study of dehydrin was only concentrated in conservative segments particularly in K segment, the roles of Y and S segments remain lacking direct evidence. In addition, 3D structure of dehydrin is not known. Therefore, the conformation research of dehydrin with bioinformatics and experimental techniques will contribute to disclose the molcecular mechanism.

The study of protective roles under stress was always limited in *in vitro* experiment. For example, the radical scavenging activity of CuCOR19 was proved in *in vitro* experiments, but it has not been demonstrated *in vivo*. The ion-binding activity was also showed *in vitro*, however, the specificity *in vivo* is different from that *in vitro*. ITP preferentially binds Fe^{3+} as well as Zn^{2+}, Cu^{2+} and Mn^{2+}, whereas only complexed with iron *in vivo*. Further work needs to be conducted *in vivo*. The physiological significance of *in vitro* cryoprotective activity of dehydrins is not clear yet. Further research is necessary to establish if the observed *in vitro* cryoprotective activity of these dehydrins is important for cryoprotection *in vivo*.

Classification of dehydrins is currently based on number and position of the three conserved motifs. However, with the increase of number and role of dehydrins found, some proteins could not be assigned to a certain class of dehydrins. For instance, chickweed leaves contain H26 protein, which has the SK_3S segment composition. It probably represents an intermediate form of proteins of SKn and KnS dehydrins. One YK_{11}-type spinach protein CAP85 shares some features of Kn, Y_2Kn and KnS classes[6]. Recently, a new classification[66], with the line of demarcation being between those Group 2 LEA proteins which are cold-tolerant versus those which are sensitive to cold stress, was reported. Dehydrins appeared to split into 3 subgroups according to this method, that is labelled 2a, 2b and 2c. Subgroup 2a had a low predicted helix content and medium to high percentage of aromatic residues, while Subgroup 2b had a high predicted loop content. All the members of Subgroup 2b had polylysine stutters, and most of those with poly-lysine stutters were found to be cold tolerant. All three subgroups were hydrophilic, but the third and smallest subgroup, Subgroup 2c, is very hydrophilic. Thus, the new classification of dehydrins is needed.

In our laboratory, the rapid accumulation of *PvSR3* mRNA on mercuric, cadmium and arsenic treated leaves was observed by Northern blot, suggesting that PvSR3 dehydrin might reduce the concentration of metal ions in cell and protect the membrane systems by ion binding activity[49]. The recombinant PvSR3 dehydrin has been expressed in *E. coli* and purified. We predict that study on the function and biochemical mechanism of dehydrin in the transgenic plant will facilitate developing and utilizing of the new crop variety, which is resistant to drought, salt, cold or heavy metals.

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