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The multifunctionality of dehydrins
An overview

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Abbreviations: IUPs, intrinsically unstructured proteins; LEA, late embryogenesis abundant

Dehydrins are highly hydrophilic proteins that accumulate during embryogenesis and water stress responses in plants. Although dehydrins were discovered in the 1980s, their physiological functions are unknown. However, recent molecular-based studies have provided insights into the multifunctionality of dehydrins. The functional versatility of dehydrins is reviewed using recent experimental evidence, and perspectives in the functional studies of dehydrins are also discussed.

Introduction

Plants respond to water stresses such as cold, drought and high salinity to survive in environmental alteration. The responses are complicated, and concomitant with physiological and molecular changes.[1-4] Late embryogenesis abundant (LEA) proteins are major accumulators in water-stressed plants.[5-9] It is believed that LEA proteins are non-catalytic proteins which protect plants from damage by abiotic stresses. LEA proteins are classified into more than seven distinct groups, with the classification depending on the nomenclature.[8] Dehydrins are group 2 LEA proteins which are also called D-11 or RAB (responsive to abscisic acid). Genetic and transgenic studies reported that the expression of dehydrins in plants enhances tolerance to abiotic stresses including low temperature, dehydration and osmotic stress,[10-18] demonstrating that at least several dehydrins participate in establishing the stress tolerance of plants. However, how dehydrins function in plants is an open question. Molecular studies have attempted to elucidate the functions of dehydrins. Because dehydrins are the most characterized LEA proteins, many reviews of dehydrins are available.[6-8,19-26] Although these reviews are useful for gaining a comprehensive understanding of dehydrins, they did not include the latest information from recent studies about the functions of dehydrins. I consider functional studies to be important for understanding how dehydrins act biologically and for speculating how dehydrin proteins and/or genes can be applied for various purposes in the fields of agriculture, biotechnology, ecology and industry.

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Properties as Intrinsically Unstructured Proteins (IUPs)

A disordered structure is a common biochemical feature of dehydrins (see reviews cited above). This feature has been documented beginning with early dehydrin studies, because amino acid sequences of dehydrins contain high proportions of Gly, charged and polar residues. Most dehydrins do not possess Cys residues and hydrophobic domains. The idea that dehydrins are IUPs is based on such characteristics upon the sequences. In fact, when native dehydrin proteins purified from plant materials and recombinant dehydrin proteins produced by *Escherichia coli* expression systems were analyzed by circular dichroism, they were judged to be disordered proteins.[27-35] It was noted that some dehydrins are rich in poly (L-Pro)-type II (PII) structures which are nonclassic secondary structures consisting of short helices.[32,33] The dehydrins remain soluble in boiling solution (see reviews cited above). The Arabidopsis dehydrins ERD10 and ERD14 showed these properties as IUPs: positive scores in some in silico prediction programs, low mobility in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, high sensitivity to proteases and narrow chemical shift dispersions in the ^{1}H-NMR spectra.[35] Solute concentrations have little influence on the disordered state of dehydrins.[34] High concentrations of potent modulators of secondary structures, such as trifluoroethanol (TFE) and SDS, could decrease the disordered state of the dehydrins and increase helicity.[29-33] This indicates that the disordered state is sufficiently stable. Because of the structural malleability, dehydrins are thought to wrap around the surface of macromolecules and fill gaps in the molecular architecture with a cohesive water layer in the cell.[36] The flexible association with various target molecules is essential to some functions of dehydrins described below, such as binding to macromolecules and protecting enzymes. The characteristics of IUPs are likely important for the functions of dehydrins.

Many IUPs are known to express specific functions by changing their secondary structures from the disordered state to the ordered one when meeting their partner molecules.[37,38] Dehydrins may also have partner molecules which can alter the secondary structures of dehydrins. Anionic phospholipids bound to a maize dehydrin DHN1. The binding was accompanied by an increase

in the helicity of the protein.[31,39] Zinc ions which bound to citrus dehydrin CuCOR15 increased the helicity of the protein and promoted the CuCOR15-nucleic acids binding.[40] These results suggest that dehydrins change their structures to bind to partner molecules such as anionic phospholipids or nucleic acids. The interconversion between disordered states and ordered ones may be how dehydrins show their functions.

Binding to Macromolecules

Close and coworkers have predicted that dehydrins might be bound to macromolecules,[19-21] because dehydrins have a unique conserved motif called a K-segment (EKKGIMDKIKEKLPG or similar sequence) which is believed to form an amphipathic helix and be related to the binding to macromolecules (see reviews cited above). Dehydrins are distributed in various compartments in the cell, including the cytoplasm, nucleus, plasma membrane, tonoplast, plastid, mitochondrion and endoplasmic reticulum (see reviews cited above). In addition, several dehydrins were extracted from membrane fractions with detergent-containing mediums.[41-43] These results suggest that at least some kinds of dehydrins can bind to membranes. The first direct evidence that a dehydrin binds to lipid vesicles was shown by Koag et al.[31] A maize dehydrin DHN1 bound to vesicles containing acidic phospholipids, such as phosphatidylserine, phosphatidylglycerol and phosphatidic acid. However, the dehydrin did not bind to vesicles of phosphatidylcholine and phosphatidylethanolamine. The phosphatidic acid vesicles increased the helicity of DHN1. Recently, the same group reported that the binding between DHN1 and acidic phospholipid vesicles required the K-segment, and the acidic phospholipid vesicles promoted the helicity of the DHN1 K-segment.[39] Arabidopsis ERD10 and ERD14 also bound to acidic phospholipid vesicles.[35] In this case, however, the secondary structures of these dehydrins were little changed by the vesicles. Since dehydrins bind specifically to acidic phospholipids, it is suggested that dehydrins may interact with the specific regions in the membrane systems of the cell.

It has also been postulated that nucleic acids may be target macromolecules of dehydrins. A Y-segment (DEYGNP or similar sequences) which exists near the amino terminus of many dehydrins was thought to be a putative nucleotide-binding domain.[20] A bioinformatic analysis of the "Protein or Oligonucleotide Probability Profile (POPP)" predicted that one of the possible functions of dehydrins is DNA binding.[44] A recent paper demonstrated that citrus CuCOR15 bound nucleic acids in a zinc-dependent manner.[40] The binding between CuCOR15 and nucleic acids was likely nonspecific, because CuCOR15 bound to both DNA and RNA with low sequence specificity. The DNA-binding domains of CuCOR15 were an H-rich domain (TTDVHHQQQYHGGEH) and a polyK-containing sequence (GGEGAHGEEKKKKKKEKKK). The former domain was shown to bind metal ions.[45] The latter domain had a sequence of EEKKKKKKEKKK, which was called a KEKE motif[46] or a charge-peptide (ChP) segment.[33] Since His was related with the metal-binding of CuCOR15,[45] His residues in the two DNA-binding domains may contribute to the zinc-dependent DNA binding of CuCOR15. Although the H-rich domain and polyK-containing sequence are not found in all dehydrins, several dehydrins possess at least one of them. This means that such dehydrins have the potential binding to nucleic acids. In addition, the conserved K-segment did not bind to DNA. These results show that DNA binding may not be a common function of dehydrins. CuCOR15 possesses no Y-segment. There has been no report showing DNA binding of the Y-segment. It would be intriguing to investigate whether the Y-segment can bind to nucleic acids.

Arabidopsis ERD10 and COR47 were identified as cytoskeleton-interacting proteins by using a mammalian fibroblasts screening system.[47] ERD10 directly bound to actin filaments and inhibited actin polymerization. ERD10 protected the actin cytoskeleton from latrunculin-mediated disruption in Nicotiana leaves. However, the binding domains of these dehydrins were not identified. Phospholipids, nucleic acids and cytoskeletons are intracellular macromolecules which are susceptible to stresses. The evidence that dehydrins bind to the macromolecules suggests that dehydrins may protect these molecules from the stresses.

Binding to Small Molecules

It is well documented that dehydrins bind calcium. Calcium binding by dehydrins is related to protein phosphorylation. The report that Rab17 was purified from maize embryo as a phosphorylated form was the first evidence of dehydrin phosphorylation.[48] After that, celery vacuole-associated dehydrin-like protein (VCaB45),[41] Arabidopsis dehydrins (ERD14, ERD10 and COR47),[42,43] and the resurrection plant (*Craterostigma plantagineum*) dehydrin CDeT6-19,[49] were detected as phosphorylated proteins. A major phosphorylation site was demonstrated to be an S-segment (LHRSGSSSSSSSEDD or related sequences).[42,43,48,50] In the case of Rab17, the S-segment, which was believed to play a role in the nuclear targeting of Rab17,[51] was phosphorylated in the nucleus.[52] Protein kinases which are related to the dehydrin phosphorylation have been discussed.[52,53] Heyen et al.[41] first reported the calcium binding of a phosphorylated dehydrin. The phosphorylated celery VCaB45 showed apparent binding to calcium, but the dephosphorylated protein did not, suggesting that phosphorylation results in an activation of calcium-binding activity. Similar results were found in ERD14, ERD10 and COR47.[42,43] Intriguingly, all of the four dehydrins, i.e., VCaB45, ERD14, ERD10 and COR47, are acidic dehydrins. However, the neutral dehydrin RAB18 did not bind to calcium even when it was phosphorylated.[43] Although a calcium-binding domain has not been identified in any acidic dehydrins, it was postulated that the calcium-binding region of these proteins may not be the S-segment itself, but upstream of the S-segment. The calcium binding of the acidic dehydrins suggests that the proteins may function as ionic buffers or calcium-dependent protein chaperones. Such functions still need to be demonstrated.

Divalent metal ions, such as zinc, manganese, nickel and cupric ions, inhibited the calcium binding of the acidic dehydrins. This means that the acidic dehydrins bind to these metals at the calcium binding site(s). The metal binding is a common function which is found not only in acidic dehydrins, but also

in neutral and basic dehydrins. Arabidopsis dehydrins (Rab18, ERD10, Xero2 and COR47),[54] *Ricinus communis* ITP,[55] and citrus CuCOR15,[45] were retained in the metal chelating columns. Since these proteins bound to metals as non-phosphorylated forms, the binding mechanism was different from the calcium-binding mechanism in the acidic dehydrins. His residues may be related to the metal binding of dehydrins because all of the dehydrins which bound to the metal chelating columns were eluted by the imidazole-containing buffers. CuCOR15, which showed the highest affinity to cupric ion, could bind up to 16 cupric ions. The non-phosphorylated CuCOR15 did not bind to calcium. A domain study of CuCOR15 suggested that a His-rich domain (HKGEHHSGDHH) was identified as a major metal-binding site of the protein.[45]

Generally, it is known that a His residue can bind metals, and the His-related residues, especially His-X_3-His and His-His, show strong affinity to metals.[45] Information about His residues in the amino acid sequences of Arabidopsis dehydrins is shown in **Table 1**. Ten dehydrins have been identified in the Arabidopsis genome.[8] All of the Arabidopsis dehydrins have more than 5 His residues in their sequences. Moreover, 7 dehydrins possess His-X_3-His and/or His-His sequences. This suggests that all of the Arabidopsis dehydrins have the potential of metal-binding without phosphorylation. Metal ions may be a common target for many dehydrins. Water stress may promote a leakage of metal ions from organelles and membranes, and may increase intracellular concentrations of free metals. It has been hypothesized that the metal binding of dehydrins may reduce various types of damage caused by free metals. This hypothesis is supported by the demonstration that transgenic tobacco plants expressing *Brassica juncea* dehydrins (*BjDHN2* and *BjDHN3*) showed more tolerance to metal stress than wild plants.[56] Intriguingly, some metal transporters possess related His-rich domains, i.e., His-rich loops, which regulate the performance of the transporters.[57,58] The His-rich regions of dehydrins may play roles in buffering metals and/or sensors of the metal level.

Dehydrin binds water. Proton NMR and differential scanning calorimetry measurements indicated that ERD10 binds a large amount of water and charged ions.[59] These researchers hypothesized that ERD10 may retain water in the drying cells to prevent protein denaturation by reducing the intracellular ionic strength.

Table 1. His-related sequences found in Arabidopsis dehydrins

Gene codes	Protein names	Amino acids	Numbers of		
			H	H-3X-H	HH
At1g20440	COR47	265	13	0	2
At1g20450	ERD10	260	11	0	0
At1g54410	-	98	13	2	2
At1g76180	ERD14	185	6	0	0
At2g21490	-	185	14	1	2
At3g50970	LTI30/XERO2	193	26	3	11
At3g50980	XERO1	128	8	0	3
At4g38410	-	163	7	0	0
At4g39130	-	151	11	0	2
At5g66400	RAB18	186	8	0	3

Prevention of Protein Denaturation

Protein denaturation is one of the most common physiological phenomena which occurring in plant cells exposed to various stresses. It may delay the efficient resumption of cellular activity when the stressed cells are recovered. In order to survive under severe stresses, protein denaturation must be prevented and the denatured proteins must be restored to functional ones. It has been well documented that dehydrins may prevent protein denaturation.

Cryoprotection of enzymes by dehydrins has been thoroughly studied. Freeze-thaw cycles provide an irreversible inactivation of enzymes such as alcohol dehydrogenase and lactate dehydrogenase. It has been reported that many kinds of dehydrins show cryoprotective activities.[30,60-67] Considering that low temperature is a major environmental stress which promotes the expression of dehydrin genes, it could be concluded that the cryoprotection is a crucial function of dehydrins. Because the cryoprotective effect of ERD10 could not be enhanced by ATP, dehydrins do not seem to correspond to ATP-dependent cold stress chaperones.[66] Two dehydrins, ERD10 and RcDhn5, efficiently prevented the lactate dehydrogenase inactivation during the freeze-thaw cycles. The preventing effects by the two dehydrins were attenuated when the K-segments were removed from the sequences, suggesting that the K-segments participate in the effects. It is likely that the amphipathic helicity of the K-segment may be related to the function.

Dehydrins act as chaperones in vitro. ERD10 and ERD14 could prevent the heat-induced aggregation and/or inactivation of several enzymes, such as lysozyme, alcohol dehydrogenase, luciferase and citrate synthase.[35] However, the mechanism of the chaperone activity is unknown. In addition, dehydrin could maintain enzyme activity under water limitation. ERD10 inhibited the reduction of lactate dehydrogenase activity during dehydration-rehydration cycles in vitro.

Although the mechanisms of the prevention of enzyme denaturation have not been confirmed, dehydrins may order water molecules around macromolecules to prevent the exposure of the hydrophobic domains to the solvent under the water stress. In case of more severe water limitation, dehydrins may prevent the structural changes of enzymes by directly interacting with the surface of the enzymes to replace the surface water.

Miscellaneous Functions

There is postulation that dehydrins may be antioxidants. Since lipid peroxidation was suppressed in transgenic tobacco in which a citrus dehydrin CuCOR19 was expressed, it was suggested that CuCOR19 may be a radical scavenger.[13] CuCOR19 protein inhibited the oxidation of soybean liposomes induced by peroxyl radicals in vitro. The effect of CuCOR19 was stronger than that of sucrose, glutathione and serum albumin. The dehydrin

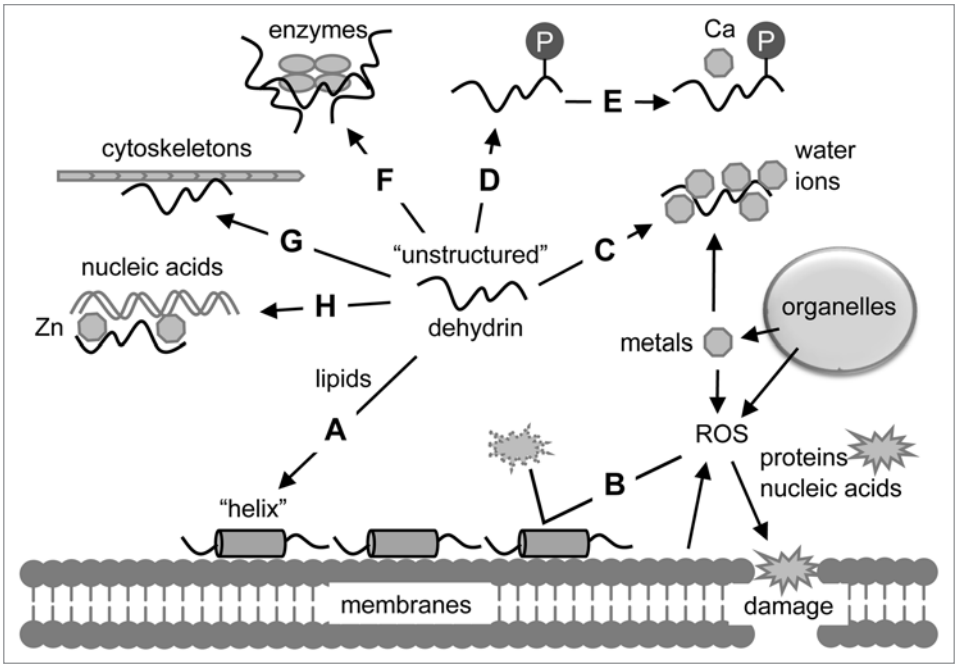

Figure 1. Functions of dehydrins with experimental evidence. Functions are represented by A–H. A, binding to phospholipids; B, radical scavenging; C, binding to water and ions; D, phosphorylation; E, binding to calcium; F, protection of enzymes; G, binding to cytoskeletons; H, binding to nucleic acids. This scheme is produced by combining data from individual studies. Some dehydrins do not have all of the functions shown in the scheme.

scavenged the hydroxyl radical and peroxyl radical, but did not superoxide anion and hydrogen peroxide.[68] Several residues of CuCOR19, such as Lys, His, Gly and Ser, may be related to the radical scavenging, because these residues were modified when the dehydrin scavenged the hydroxyl radical. Dehydrins may protect cellular components from oxidative stresses. Recently, modification of dehydrins by radicals was detected in vivo. When sunflower seeds were imbibed in water, a dehydrin in non-dormant axes was carbonylated.[69] The oxidative modification of dehydrins may be related to an alleviation of embryonic dormancy.

It was reported that an intracellular ERD10 affected bacterial growth.[70] When ERD10 protein was overexpressed in *Escherichia coli*, the bacterial growth was inhibited. Truncation experiments suggested that K-segments of ERD10 are related to the inhibitory effect. It has not been demonstrated whether or not ERD10, which is applied to the culture medium, shows antimicrobial activity. The physiological role of the antimicrobial activity of ERD10 is unknown.

Conclusion and Perspective

Figure 1 represents a scheme of the in vivo functions of dehydrins which were hypothesized from the experimental evidence. As shown, dehydrins actually show multifunctionality, whereas all the dehydrins do not necessarily have all of the functions. It is necessary to identify which functions are common in many dehydrins and which are restricted to specific dehydrins. At present, binding to acidic phospholipids, binding to ions, and cryoprotection are candidates of the common functions. Because dehydrins are IUPs, functional domains or sequences can act without structural limitation under the various physicochemical environments. Thus, domain studies are efficient for elucidating the mechanisms of the functions. If the correlations between domains and functions are clarified, the functions of many dehydrins can be postulated from their domain constructions.

Many functions of dehydrins have been demonstrated by in vitro experiments. However, in vitro experiments are not sufficient to confirm the physiological functions of dehydrins, because it is supposed that the functions in vitro are not always expressed in vivo. Thus, studies to understand how the in vitro functions affect growth and stress tolerance in plants are needed. One example is to investigate physiological differences between transgenic plants expressing a mutant dehydrin lacking target domain(s) showing specific function(s) and the plants expressing wild dehydrin. If any physiological differences are found, the direct relationships between the domain and the corresponding physiological role(s) can be elucidated. Information from such studies will provide new strategies for efficiently breeding stress-tolerant plants using dehydrin genes.

Generally, dehydrins are believed to interact non-specifically to partner molecules. In fact, dehydrins bind to various molecules such as water, ions, phospholipids, proteins and nucleic acids. However, in most cases, the direct binding between dehydrins and target molecules was assessed without any mediating factors, except the binding between CuCOR15 and nucleic acids, which was dependent on zinc. If appropriate co-factors are thus supplied to the assay system, more kinds of interactions can be detected. Finding such co-factor-mediated interactions could be the breakthrough needed to find unidentified functions of dehydrins. Functional studies of dehydrins are beginning to be conducted.

These studies may enable the design of super dehydrins whose functions are strengthened, and provide crucial data needed to utilize dehydrins in various fields.

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