Hiding in plain sight: the F segment and other conserved features of seed plant $\text{SK}_n$ dehydrins

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Abstract

Main conclusion An 11-residue amino acid sequence, DRGLFDLGKK, is highly conserved in a subset of dehydrins found across the full spectrum of seed plants and here given the name F-segment.

An 11-residue amino acid sequence, DRGLFDLGKK, is highly conserved in identity and polarity in 130 non-redundant dehydrin sequences representing conifers and all major angiosperm groups. This newly described motif is here given the name F segment based on the pair of hydrophobic F residues at the core of the sequence. The majority of dehydrins previously classified as $\text{SK}_n$ dehydrins contain one F segment N terminal to the S and K segments and can accordingly be reclassified as $\text{FSK}_n$ dehydrins. A cysteine-containing variant, GCGMFDLKK, occurs in a few rosid and asterid taxa. The S segment in this and other dehydrin types also includes previously overlooked conserved features, including a KLHR prefix and charged or G residues within and following the characteristic string of S residues. Secondary structure prediction models indicate that the F segment and S segment prefix may form amphipathic helices that could be involved in membrane or protein binding.

Keywords LEA protein · Localization · Membrane binding · Phosphorylation · Sequence conservation

Introduction

Dehydrins are a family of land plant proteins that may be expressed constitutively at low levels but are often produced de novo or at higher levels in response to drought, low temperature, or other stresses. They have been detected by western blotting or nucleic acid sequencing in all types of land plants, including bryophytes, pterophytes, gymnosperms, and all major groups of angiosperms. The main structural features of dehydrins were first described over 20 years ago (Close 1996), and include the number and modular arrangement of three types of short, distinctive segments in the protein, designated K, Y, and S segments. Using the YSK shorthand nomenclature proposed by Close, most known dehydrins fall into five types: $\text{K}_n$, $\text{SK}_n$, $\text{K}_n\text{S}$, $\text{Y}_n\text{K}_n$, and $\text{Y}_n\text{SK}_n$.

With a very few exceptions (noted below) the common feature of all dehydrins is one or more repeats of the K segment, a lysine-rich, 15 amino acid sequence with a highly conserved pattern of charged and nonpolar residues. The consensus K segment sequence in angiosperms is EKK-DIMGKIKEKLPG, with a generally similar pattern conserved in conifer variants (Jarvis et al. 1996; Perdiguero et al. 2014). The identity or polarity of all K segment residues is highly conserved. The K segment forms an amphipathic $\alpha$-helix in nonpolar environments and binds membranes (Koag et al. 2003, 2009; Eriksson et al. 2011), suggesting that a primary function of dehydrins is to protect membranes and perhaps proteins against dehydration stress.

The Y segment comprises seven residues with the consensus sequence (V/T)DEYGNP. When present, usually one to three closely spaced copies of the Y segment are located towards the N terminal end of the protein relative to the S or K segments. A group of four *Coronaria sericea* dehydrins contain from 14 to 35 recognizable Y segments.
Majority of SKn dehydrins contain a single copy of this consensus DRGLFDFLGKK. Here I report that the longer conserved sequence with the preliminary 11 residue N-terminal end of the protein. Further inspection suggested a trend in both FSKn and YnSKn dehydrins. Note additional sequence conservation around the S segment or at the C-terminus. Dehydrins with S segments are overrepresented in NCBI by registration of dozens of allelic variants and nearly identical homologous sequences in closely related taxa. Based on the number and occurrence of K, Y, and S segments, the resulting 395 sequences were classified into the five types described by Close, with the few exceptions noted. It was in this process that I first noticed the F segment.

After a BLAST search in the NCBI database for proteins containing variations of the 11-residue sequence DRGLFDFLGKK, I screened the results as above to remove non-dehydrins and redundant variants within taxa. Sequences were aligned using Clustal Omega and the alignments checked and adjusted manually. To explore sequence variation in and around the conserved segment, I isolated and aligned a 32-residue sequence centered on it and tallied the amino acids of each type found at each position.

To explore variation in and around the S segment, all S-segment containing dehydrins from the library were extracted. I excerpted a 35-residue segment centered on the consecutive S residues. These excerpts were manually aligned using the first S residue, recognizable variants of a commonly occurring HR prefix, and the first occurrence of a charged residue, usually D or E, following the S residues. Alignment of the latter was forced by inserting blanks in sequences with fewer than the maximum number of nine S residues. Following alignment, I tallied the amino acids at each position for each of the four dehydrin types (including FSKn) that incorporate S segments.

The eight-structure class protein secondary structure prediction model SSpro8 (Magnan and Baldi 2014) was run on a selection of full-length FSKn dehydrin sequences to assess the potential for helical or sheet structures in and around the F and S segments.

Results and discussion

Frequency of the F-segment in dehydrins

In the initial screening and classification of 395 minimally redundant dehydrin sequences recovered from NCBI, 93 were classified as SKn type dehydrins based on the presence of S and K but no Y segments (Table 1). I found F segments in 82 of these, indicating that most known SKn type dehydrins can be reclassified as the FSKn type. There were only nine FKn type dehydrins in the sample, and only one with more than one copy of the F segment, an F2SK2 dehydrin in Rhododendron (AGI36547.1). There were no

Materials and methods

To compile a library of dehydrin sequences, I searched the NCBI data for sequences identified as dehydrins and conducted BLAST searches on the angiosperm and conifer consensus K segment sequences. The results were screened to identify and remove sequences lacking core dehydrin characteristics, allelic variants, and highly similar homologs within taxa. Genera such as Pinus, Vitis, and Solanum are overrepresented in NCBI by registration of dozens of allelic variants and nearly identical homologous sequences in closely related taxa. Based on the number and occurrence of K, Y, and S segments, the resulting 395 sequences were classified into the five types described by Close, with the few exceptions noted. It was in this process that I first noticed the F segment.

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sequences with both Y and F segments. An additional 75 sequences that were incomplete at the N terminal end could potentially be any of a number of types including FKn or FSKn type dehydrins. About 25% of the 72 complete gymnosperm dehydrin sequences in the library contained F segments, but none contained Y segments (Table 1). The absence of Y segments in gymnosperms was noted by Perdiguero et al. (2012, 2014), but not in earlier reviews of dehydrin occurrence, structure, and function (e.g. Close 1996; Allagulova et al. 2003; Rorat 2006; Eriksson and Harryson 2011). There were also no complete gymnosperm SKn or KnS dehydrins in the curated library, so that the available data indicates that in gymnosperms the S segment is always preceded by an F segment.

Conservation variation in the F segment

The BLAST search of the NCBI database for proteins containing variations of the 11-residue sequence DRGLFDFLGBK yielded an initial library of about 575 complete and partial sequences, about 300 of which were readily recognized as dehydrins based on the presence of one or more recognizable variants of the K segment. After screening out redundant sequences, I arrived at a list of 130 minimally redundant proteins representing a broad spectrum of seed plant groups, including conifers as representative of gymnosperms and a variety of groups within the monocot, caryophyllid, rosid, and asterid clades of angiosperms. 123 of these also contained an S segment.

Conservation variation in the S segment

The S segment has been previously characterized as a string of three to as many as 13 serine residues, and typically followed by a string of charged K, D, or E residues (Eriksson and Harryson 2011). There were also no complete gymnosperm SKn or KnS dehydrins in the curated library, so that the available data indicates that in gymnosperms the S segment is always preceded by an F segment.

Additional conserved features around the S segment emerge on closer examination. In dehydrins with S segments on the N terminal side, the first S in the sequence is typically followed by a D, E, or G residue, which is then followed by two to as many as 12 additional serine residues. In KnS dehydrins, this is mirrored by a highly conserved terminal DSD motif, which, in the 25 KnS sequences in my curated sample, always forms the C-terminus of the protein. In FSKn dehydrins, there is a well-conserved KLHR prefix (Fig. 2), and the C terminal S is followed by 2–7 acidic residues, giving the generalized sequence KLHRS(D/E)S2–12(D/E)2–7, followed by a string of predominantly G and charged residues. The prefix is somewhat less conserved in YnSKn and SKn dehydrins, where the initial K is not conserved and a polar residue may replace H. KnS dehydrin S segments have a more variable mix of charged, H, and G residues as a prefix.

Table 1

| Dhn type | Angiosperm | Gymnosperm | Total | % of total |
|----------|------------|------------|-------|------------|
| Kn + SKn | 37         | 38         | 75    | 19.0       |
| Kn       | 27         | 9          | 36    | 9.1        |
| KnS      | 25         | 0          | 25    | 6.3        |
| SKn      | 11         | 0          | 11    | 2.8        |
| FKn      | 3          | 6          | 9     | 2.3        |
| FSKn     | 65         | 17         | 82    | 20.8       |
| YnKn     | 37         | 0          | 37    | 9.4        |
| YnSKn    | 113        | 0          | 113   | 28.6       |
| Uncommon types | 5 | 2 | 7 | 1.8 |
| Total    | 323        | 72         | 395   |            |

‘‘?’’ indicates incomplete N terminal sequences lacking F and Y segments
Predicted structures from SSpro8 (Magnan and Baldi 2014) for a selection of FSKn dehydrins consistently suggest a short helical region centered on the five residue core sequence LFDFL in the consensus F segment and the GCG variant, including those where the D is replaced by a G residue. In a helical wheel projection of this short sequence, the four hydrophobic residues are arrayed on one side of the helix, with the charged D or somewhat hydrophilic G residue opposing them. This observation suggests that the F segment could have amphiphilic membrane or protein binding properties similar to those of the K segment. The same model also consistently predicts helical regions N terminal to the S segment. The conserved pattern of hydrophobic residues in this region (Fig. 2) could result in an amphiphilic structure here as well. All K segments are also consistently helical in the SSpro8 predictions.

**Fig. 1** Alignment, conservation, and variation in 32-residue aligned sequence excerpts from selected seed plant taxa centered on the F segment in FSKn dehydrins. The “i” in the numbered residue positions in the top row indicates a presumed insertion in Poaceae. Residues are ordered and colored by Kyte-Doolittle hydrophobicity (Lefranc 2017). The F segment is outlined in black.

**Structural modeling**

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Conservation implies function

The high degree of conservation in the K, Y, F, and S segments in these otherwise highly variable and unstructured proteins implies that all four segment types play important roles in the overall function of the protein. Functional studies show that the K segment binds membranes and therefore suggest that the main function of dehydrins is membrane protection (Koag et al. 2003, 2009). Membrane binding may be modulated by phosphorylation of S and pH-dependent dissociation of H residues (Eriksson et al. 2011), suggesting a function for the conserved H and S residues in the S segment.

The functions of the Y segment remain a mystery. Close et al. (1996) found some similarity between the Y segment and a nucleotide binding site in bacterial chaperones, an observation that has been echoed in some reviews (e.g. Allagulova et al. 2003; Rorat 2006). That similarity is not at all obvious on inspection of the sequences in the original report (Martin et al. 1993), and in any case there seems to have been no follow-up on this assertion. Whatever its function, gymnosperms are able to survive in a wide range of environments, including extremely dry or cold conditions, without any apparent need for the Y segment.

As noted above, the F segment may form a short, amphipathic helix with membrane or protein binding properties, and the S segment prefix may also have amphipathic properties. Sucrose, raffinose, and various compatible solutes have been hypothesized to stabilize (Carpenter and Crowe 1988) or replace (Clegg 1985) hydration shells around proteins or membranes; perhaps dehydrins play a similar role. Alternatively, binding by K segments or other amphipathic regions could anchor dehydrins to membranes or proteins so that they could act as "molecular spacers" (Strimbeck et al. 2015), preventing close approach and conformational or phase changes associated with repulsive forces under dehydration (Wolfe and Bryant 1999). Protein modification and binding studies (e.g. Koag et al. 2003, 2009; Eriksson et al. 2011) or other protein modification experiments may help clarify the binding and related protective properties of the F segment and other conserved regions of the different dehydrin types.

Author contribution statement GRS conducted all of the original analyses reported in this article.

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