Desiccation stress of entomopathogenic nematodes induces the accumulation of a novel heat-stable protein

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SUMMARY

The present study describes a novel heat-stable, water-stress-related protein with a molecular mass of 47 kDa (designated Desc47) in the entomopathogenic nematode *Steinernema feltiae* (IS-6). The protein was accumulated about 10-fold (from 7·84 ± 1·85 to 74·09 ± 4·35 % relative content level [RCL]) in dehydrated clumps of infective juveniles (IJs), which had lost 34·4 % of their initial water content (from 65·1 ± 1·7 % to 42·7 ± 0·72 %) in a desiccation-tolerance-inducing treatment (97 % relative humidity [RH] for 3 days). The appearance of Desc47 was accompanied by trehalose accumulation (from 300 to 600 mg trehalose/g protein) during the process of inducing the IJs into a quiescent anhydrobiotic state. A second cycle of IJ dehydration did not alter the RCL of Desc47 (79·3 % for the first cycle and 73·3 % for the second cycle). Desc47 retained its high RCL (69·7 %) in rehydrated active IJs for 3 days, reaching 51·2 % of its initial RCL only after a week. No homology to other known proteins was found by mass-spectrometry electrospray-ion-trap analysis. However, of the 5 sequences obtained from the protein (ranging from 11 to 21 amino acids), the 21-amino-acid peptide N V A S D A V E T V G N A A G Q A G (D/T) A V showed excellent homology (74 % identity in 19 amino acids) to the cold-responsive protein COR14b (g6564861) from *Triticum aestivum*. In the *Caenorhabditis elegans* predicted proteome database search, the N21 yielded the first-best identity score (59 % identity in 17 amino acids) to the CE-LEA homologue protein (g2353333). In plants, COR and LEA are related proteins, heat-stable, which are expressed in response to both dehydration and cold acclimation. The implication of the involvement of Desc47 and the osmoprotectant trehalose in the desiccation-tolerance mechanisms of *S. feltiae* is discussed.

Key words: anhydrobiosis, water-stress-related protein, desiccation tolerance, *Steinernema feltiae*.

INTRODUCTION

Entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae have been known for decades (Poinar, 1990). These nematodes can actively locate, infect and kill, with the co-operation of a symbiotic bacterium (*Xenorhabdus* spp. and *Photorhabdus* spp., respectively), a wide range of insect species. Both *Steinernema* and *Heterorhabditis* pass through 4 juvenile stages in the insect host before maturing. Only the infective juveniles (IJs) can survive outside the insect host and move from one insect to another. These parasitoid nematodes are considered among the most promising alternatives to chemical control of insect pests (Georgis & Manweiler, 1994). However, their sensitivity to desiccation stress and other environmental constraints reduces their field efficacy (Kaya & Gaugler, 1993).

Free-living stages of some species of nematodes can survive desiccation stress in a state of anhydrobiosis (Womersley, 1987). Anhydrobiosis is a general term for a reversible, physiologically arrested state of dormancy that results from water loss. The following traits broadly define the characteristics of cryptobiotic nematodes (Barrett, 1991): they can lose up to 95–98 % of their body water and in anhydrobiosis they have virtually no metabolism, thereby conserving energy reserves. Steinernematidae and Heterorhabditidae have been classified by Womersley (1990) as slow-dehydration strategists which are only capable of a dormancy described as quiescent anhydrobiosis.

In a previous study, we isolated a desiccation-tolerant strain of *Steinernema feltiae* (IS-6) from a desert region in Israel, and determined the anhydrobiosis-inducing conditions affecting its desiccation survival (Solomon, Paperna & Glazer, 1999). In that study, we demonstrated that strain IS-6 IJs can survive low humidity after being induced into a quiescent anhydrobiotic state at 97 % relative humidity (RH) for 3 days. Womersley (1987, 1990) and Barrett (1991) reviewed the importance of slow rates of water loss in different species of nematodes in modulating metabolic and biochemical processes crucial for the successful induction of a state of anhydrobiosis. However, very little is known on the

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physiological changes which occur in nematodes in response to water loss. The best-documented biochemical change in anhydrobiotic organisms, including nematodes, is the accumulation of trehalose during the dehydration process (Womersley, 1981, 1987, 1990; Crowe & Crowe, 1992). Trehalose is known to be accumulated to high concentrations by organisms that naturally survive dehydration, with specific properties that protect membranes and proteins against desiccation in a model system (Crowe & Crowe, 1992).

Water-stress-related proteins have been extensively described in plants and in the cyanobacterium *Nostoc commune* (Baker, Steele & Dure, 1988; Scherer & Potts, 1989; Hill *et al*. 1994). To our knowledge, nothing is known in nematodes or other anhydrobiotic animals about water-stress-related proteins involved in the anhydrobiotic process.

In a preliminary study, unsuccessful attempts were made to identify water-stress-related proteins from the total protein extract of dehydrated nematodes. In plants, water-stress-related proteins are known to be highly hydrophilic and resistant to coagulation by heat (Close, Kortt & Chandler, 1989; Thomashow, 1998), therefore, it seemed worthwhile to use the heat-stable protein fraction from dehydrated nematodes as a purification step for polypeptides that are potentially responsive to water loss.

The present study is the first description of a heat-stable, water-stress-related protein which is accumulated in nematodes entering the anhydrobiotic state, and demonstrates the association between water loss and accumulation of the osmoprotectant trehalose in the dehydration process of the entomopathogenic nematode *S. feltiae* (IS-6).

MATERIALS AND METHODS

Nematode culture

Steinernema feltiae (IS-6) (Solomon *et al*. 1999) were reared in the last instar larvae of *Galleria mellonella* as described elsewhere (Dutky, Thompson & Cantwell, 1964). The emerging IJs were stored for 3–4 weeks in 250 ml of distilled water in culture flasks at 5 °C prior to use in the experiments.

Desiccation of nematodes

Aggregates (clumps) of IJs (0·2 g) were placed on the surface of Petri dishes (5 cm diameter) and the superficial water was removed with the edge of a Whatman filter paper. They were then dehydrated in 97 % RH (desiccation-tolerance-inducing treatment) for different periods as described elsewhere (Solomon *et al*. 1999). After dehydration, the collected clumps were kept frozen in liquid nitrogen until use for the experiments. The 97 % RH was controlled in sealed desiccators with 60 ml of saturated K_2SO_4 solution at 23 °C (Winston & Bates, 1960). Two additional experimental groups were tested: untreated nematodes (control group) kept in water for 3 days and post-desiccated IJs that were rehydrated in water for 3 and 7 days at 23 °C.

Heat-stable protein extraction

The fraction of heat-stable proteins was extracted from frozen clumps of IJs as described by Pelah, Shoseyov & Altman (1995). The nematodes were homogenized in liquid nitrogen to a very fine powder with a chilled mortar and pestle in extraction buffer (50 mM Tris–HCl, pH 7). The buffer contained water-insoluble PVP (polyvinylpolypyrrolidone) (1 % w/v) and a cocktail of protease inhibitors (EDTA [ethylenediaminetetraacetic acid] and PMSF [phenylmethylsulfonyl fluoride] to final concentrations of 5 mM). Homogenates were centrifuged (14000 ***g*** for 15 min at 4 °C) and the supernatant fraction was collected and boiled for 10 min, kept on ice for 5 min and centrifuged again at 14000 ***g*** for 3 min at 4 °C. The uncoagulated proteins in the supernatant (200 μl) were precipitated in cold acetone (1000 μl) at −20 °C for 2 h and then centrifuged at 14000 ***g*** for 15 min at 4 °C. The pellet was boiled at 100 °C for 5 min in SDS–PAGE sample buffer (60 mM Tris–HCl, pH 6·8, 2 % SDS, 25 % (v/v) glycerol, 0·1 % bromophenol blue, and 14·4 mM 2-mercaptoethanol) (Laemmli, 1970). Protein quantity determination in each sample was performed with Coomassie brilliant blue (CBB R-250, Sigma) on filter paper according to the method described by Ghosh *et al*. (1988) with modifications (Salomon, 1989) using bovine serum albumin (BSA, Sigma) as a standard. The dye was eluted from the spots in 200 μl of 1 % SDS by gentle shaking for several hours. Protein concentration was determined by absorbance at 570 nm in an automated ELISA reader (Salomon, 1989).

Gel electrophoresis of proteins

Proteins were separated by SDS–PAGE (Laemmli, 1970) in 8–20 % gradient polyacrylamide gels. Each lane was loaded with equal amounts of protein and separation was at 150 V for 180 min in a Bio-Rad mini gel apparatus. Gels were stained for 1 h with 1 % (w/v) CBB R-250 in 45 % (v/v) methanol and 10 % (v/v) acetic acid and destained for several hours in 10 % methanol and 10 % acetic acid.

Quantitation of Desc47 and molecular weight determination

Relative content levels (RCL %) of polypeptides (bands) were analysed by the NIH Image 6.1/fat software program from the Coomassie-stained gels, scanned on a standard scanner (Umax). The RCL of Desc47 was calculated from the number of pixels

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(intensity per constant area) of the selected band, divided by the value of pixels of an adjacent unchanged constitutive band with a molecular mass of 52 kDa (internal marker).The molecular weight in kDa was calculated by the NIH program by comparing to low-range standard marker values (Bio-Rad, Israel).

Mass-spectrometry analysis of Desc47

Mass-spectrometry electrospray-ion-trap analysis of Desc47 was performed at the Protein Center of the Department of Biology, Technion-Israel Institute of Technology. Approximately 1 μg of Desc47 was digested with trypsin overnight at 37 °C. The resulting peptides were resolved by reverse-phase HPLC and microsprayed directly into the electrospray-ion-trap mass spectrometer (LCQ, Finnigan). The collected data were compared to simulated proteolysis and fragmentation of known proteins in the 'owl' database using the Sequest software (J. Eng & J. Yates, University of Washington, USA). Further amino acid identification of Desc47 was performed in 4 fragment residues obtained (ranging from 11 to 21 amino acids) by sequencing in a peptide sequencer (Procise, Perkin Elmer). Homology of these short fragments to known proteins was explored using WUBLAST2 software to search the EMBL database and in *Caenorhabditis elegans* predicted proteome database from the Sanger Center, Cambridge, UK.

N-terminal amino acid sequencing

Gel slices of Desc47 were re-resolved in 8–20 % polyacrylamide gradient gels as already described. Following electrophoresis, the gel was soaked in transfer buffer (10 mM CAPS in 10 % methanol, pH 11) for 5 min while the Immobilon membrane (PVDF, Millipore) was rinsed in 100 % methanol and stored in transfer buffer. The protein was electrotransferred to the membrane using a semi-dry blot transfer system (Bio-Trans midi, Gelman Sciences) and blotted for 2 h (1 mA/cm^2) at room temperature. Then the membrane was washed in deionized water for 15 min, stained with 1 % (w/v) CBB R-250 in 50 % methanol and destained (50 % methanol, 10 % acetic acid) for 10 min at room temperature. The membrane was rinsed in deionized water, air-dried and stored at −20 °C prior to use, as described elsewhere (Matsudaira, 1987). The stained bands were cut out from the membrane and processed by peptide sequencer (Procise AT 491, Perkin Elmer) for sequencing of the N-terminal region. Homology of the N-terminal region to known proteins was determined using WUBLAST2 software to search the EMBL database and in *Caenorhabditis elegans* predicted proteome database from the Sanger Center, Cambridge, UK.

Peptide mapping

Peptide mapping analysis was performed with a Promega fingerprinting kit. Gel slices of Desc47 and a polypeptide extracted from the rehydrated IJs, showing identical mobilities, were equilibrated in running buffer for 30 min at room temperature. The gel slices were loaded into the wells of the stacking gel and covered with 5 μl of gel slice overlay solution, and 5 μl of endoproteinase Glu-C (cleaves at the carboxylic side of glutamic acid residues) diluted 1:20. Two additional wells served as controls: one containing phosphorylase B as a control protein and one containing only the applied protease. The proteins were separated by SDS–PAGE in 15 % polyacrylamide. An initial voltage of 100 V was applied until the proteins were stacked at the interface between stacking and resolving gels. Then enzymatic cleavage was allowed for 20 min, followed by electrophoresis at 200 V until the dye front reached the gel bottom. The polypeptide fingerprinting pattern was visualized by CBB staining.

Water loss measurements

Water contents of clumps exposed to 97 % RH were determined by a gravimetric method: the fresh weight of the clumps was determined on an analytical balance (Sartorius Basic, Germany) after exposure to 97 % RH for 4, 8, 12, 48 and 72 h, then kept frozen in liquid nitrogen until use. The water contents (%) of the frozen samples were calculated on the basis of their dry weights following lyophilization in a Cherist L-1 lyophilizer (Germany) at −56 °C for 24 h. Water contents in the desiccation experiments were determined from 3 independent replicates.

The water content of single IJs was determined from 10 individuals by quantitative interference microscopy (Ellenby, 1968). The IJs were desiccated on a glass slide in a sealed desiccator at 97 % RH for 3 days, then immediately covered with liquid paraffin and photographed under a Vickers M41 photoplan interference microscope equipped with a fringe eyepiece and a Zeiss micro-flash. Measurements of the fringe displacement and specimen diameter were obtained from the photographs of the anterior region of the nematode. The water content (%) was calculated using the method described by Perry (1977).

Extraction and enzymatic hydrolysis of trehalose

Trehalose was extracted from frozen clumps of IJs after exposure to 97 % RH for 4, 8, 24, 48 and 72 h. Each sample was boiled for 10 min in 1 ml of distilled water at 100 °C following homogenization in liquid nitrogen with a mortar and pestle to a very fine powder. The homogenates were centrifuged (14000 ***g*** for 30 min at 4 °C) and the supernatant

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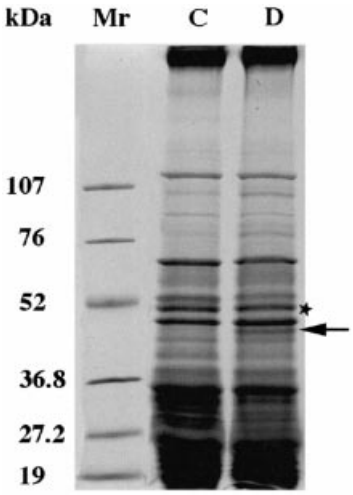

Fig. 1. SDS–PAGE (8–20 % acrylamide gradient gel) profile of heat-soluble, uncoagulated proteins. C, Control non-desiccated nematodes kept in distilled water for 3 days at 23 °C. D, Desiccated clump of IJs that lost 34·4 % of its initial water content after exposure to 97 % RH for 3 days. Molecular markers (Mr) are shown on the left. Each lane was loaded with 40 μg heat-soluble proteins. The arrow indicates Desc47 and the asterisk marks the constitutive protein ($\sim$ 52 kDa) used as an internal marker that was unchanged by desiccation.

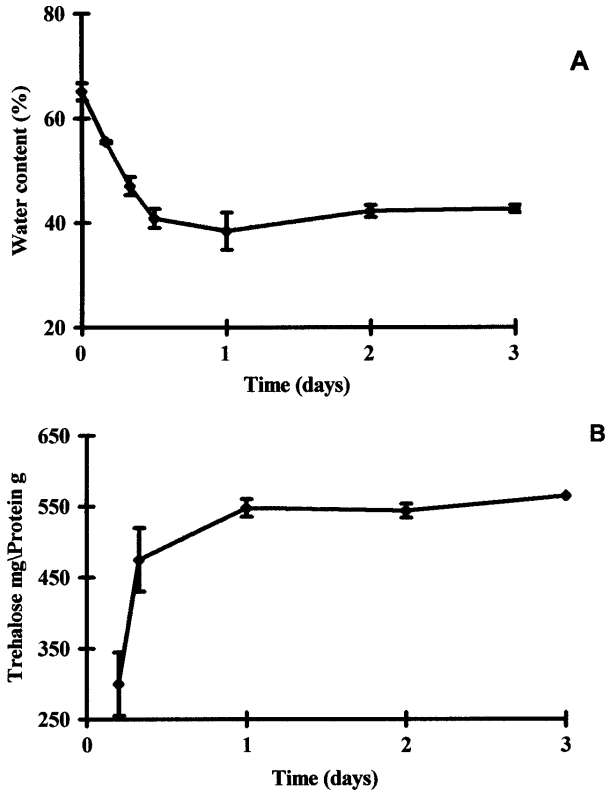

Fig. 2. (A) Water loss in clumps of *Steinernema feltiae* (IS-6) during 3 days exposure to 97 % RH ($n = 3$, error bars show $\pm$S.D.). (B) Accumulation of trehalose in response to water loss in *S. feltiae* (IS-6) IJs ($n = 3$, error bars show $\pm$S.D.).

fraction was stored in liquid nitrogen until needed for the enzymatic reactions. Protein concentration in each sample was determined by the method of Lowry *et al*. (1951) using BSA (Sigma) as a standard. Protein denaturation and solution clarification were performed on each supernatant before the enzymatic hydrolysis, with Carrez-I solution (3·6 % potassium ferricyanide) and Carrez-II solution (7·2 % zinc sulfate) in 0·5 M NaOH, pH 7·8 (Boehringer-Mannheim, 1986). Trehalose was assayed with trehalase (Sigma) as follows: 50 μl of extract was incubated for 1 h at 30 °C in a total volume of 250 μl with 150 μl of 25 mM sodium acetate, pH 5·7 and 0·27 U/ml trehalase. Trehalose concentration in each sample was calculated from the formed glucose and was quantified per gram of total water-soluble protein. Glucose was quantified using a glucose oxidase-peroxidase kit (Sigma). In calculating the trehalose concentration, corrections were made for the glucose concentration in the non-enzymatically treated supernatant fraction.

Trehalose levels were determined from 3 independent experiments and expressed as weight per soluble protein weight because of the unchanged protein content per dry weight of desiccated and control non-stressed nematodes ($29 \pm 0{\cdot}67$ *versus* $30{\cdot}6 \pm 1{\cdot}65$ μg protein/mg dry weight).

RESULTS

Identification of a water-stress-related protein in IJs

A heat-stable (uncoagulated) water-stress-related protein with a molecular mass of 47 kDa (designated Desc47) was recovered in dehydrated clumps of IJs (Fig. 1), which had lost 34·4 % of their water content (from $65{\cdot}1 \pm 1{\cdot}7$ % to $42{\cdot}7 \pm 0{\cdot}72$ %) (Fig. 2A). This water-stress-related protein was induced to about 10-fold its level in nonstressed nematodes (from $7{\cdot}84 \pm 1{\cdot}85$ to $74{\cdot}09 \pm 4{\cdot}35$ % RCL). A second cycle of dehydration did not alter the RCL of Desc47 (79·3 % RCL for the first cycle and 73·3 % for the second cycle). The induction of Desc47 was accompanied by trehalose accumulation during the process of inducing the IJs into a quiescent anhydrobiotic state (Fig. 2B).

Proteolytic peptide mapping of Desc47 from desiccated and rehydrated nematodes yielded the same SDS–PAGE peptide pattern of 3 major polypeptides at 46, 45 and 37 kDa (not shown), indicating that these proteins from the desiccated and rehydrated nematodes are identical. Desc47 retained its high content level in the rehydrated active IJs for 3 days (69·7 %, RCL), declining to 51·2 % of its initial RCL only after 1 week.

Amino acid sequence analysis of Desc47

No homology to other known stress proteins was found, using the mass-spectrometry method. Further

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Table 1. Amino acid sequences of 5 peptides from the water-stress-related protein Desc47

(Amino acids placed in parentheses represent the 2 possibilities for that amino acid.)

Location in Desc47	Amino acid sequences (name of peptide)*
N-terminal region	S (Q/R) T D D I S N (T/L) (A/N) R F (A/H) A – (S14)
	G N A A G T A V X S A – (G11)
Internal segments	V D H X A N D A V D A P R (K/Q) N A – (V16)
	X T A A S (I/G) A E X V S D (G/A) A D L (N/V) – (X17)
	N V A S D A V E T V G N A A G Q A G (D/T) A V – (N21)

* X represents an undetermined amino acid.

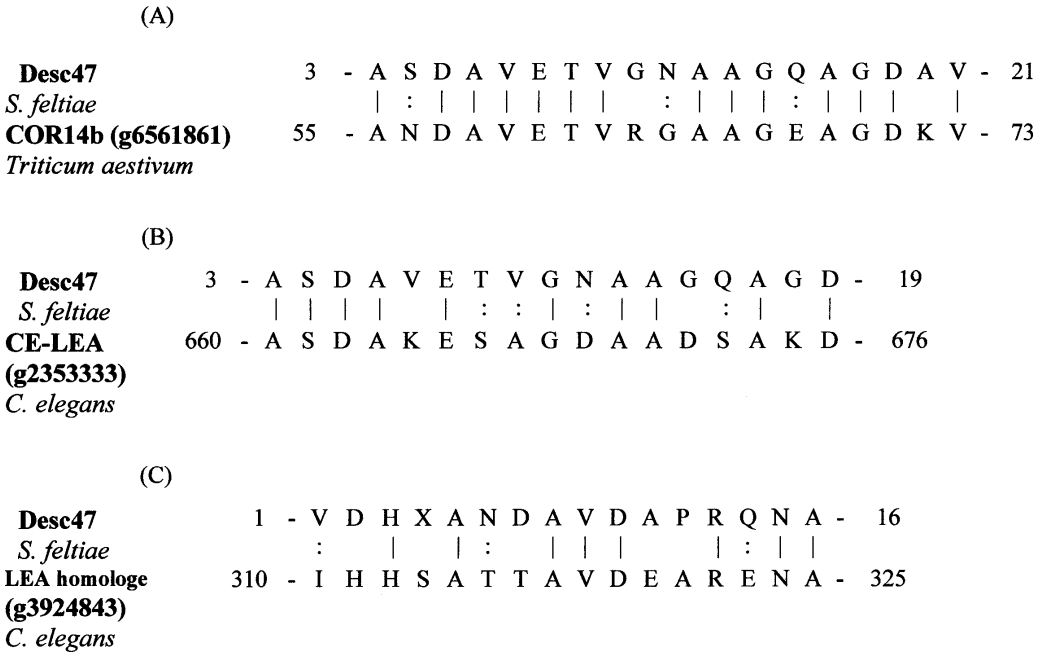

Fig. 3. Sequence alignment of the N21 peptide with the amino acid sequence of the cold-responsive protein COR14b from *Triticum aestivum* (A) and comparison of N21 (B) and V16 (C) peptides to the *Caenorhabditis elegans* LEA predicted homologue proteins. The symbol (|) represents identical amino acids; (:) represents similar amino acids.

identification of Desc47 was performed with 4 short cleaved internal fragments containing 11 (G11), 16 (V16), 17 (X17) and 21 (N21) amino acid (aa) residues, and an N-terminal fragment of 14 aa residues (S14) (Table 1). However, when using such short amino acid fragments care needs to be taken in interpreting the results of the database similarity search.

Among these peptides, N21 gave first-best alignment with excellent homology (74 % identity in 19 aa) to the cold-responsive protein COR14b (g6561861) from wheat (*Triticum aestivum*). In the *C. elegans* predicted proteome database search, the N21 and V16 fragments yielded first-best identities (High Score = 44 and 41, respectively) to 2 distinct LEA homologue proteins. N21 exhibited 59 % identity in 17 aa to the CE-LEA homologue protein (g2353333) of the 733 predicted aa, while V16 exhibited 50 % in 16 aa to another LEA protein homologue of 470 predicted aa (g3924843) (Fig. 3).

Water content of clumps and single IJs

Similar water content results were obtained using gravimetric and interference microscopy methods: fresh clumps contained $65{\cdot}1 \pm 1{\cdot}7$ % water, similar to the results of fresh individual IJs ($69 \pm 3{\cdot}58$ %) measured by interference microscopy. When both clumps and individual IJs were exposed to 97 % RH for 3 days, they lost the same relative amounts of water (34·4 % *versus* 40 %).

DISCUSSION

In the present study we show that during adaptation to desiccation stress, a novel heat-stable protein (Desc47) is accumulated in the entomopathogenic nematode *S. feltiae*. To the best of our knowledge, this is the first report of a direct association between water loss and accumulation of a water-stress-related protein in nematodes or other anhydrobiotic animals.

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Excellent homology was found between the major internal fragment of Desc47 (N21) and the COR14b protein from wheat (*T. aestivum*). In addition, in the *C. elegans* predicted proteome database search, 2 different fragments (N21 and V16) of Desc47 scored the highest identity to 2 different LEA homologue proteins. It is reasonable to assume that the other amino acid fragments of Desc47 would not match the COR or the LEA proteins since they fall outside the conserved regions.

In plants, COR and LEA are related proteins, heat-stable, which are expressed in response to both dehydration and cold acclimation (Neven *et al*. 1993; Close, 1996, 1997; Thomashow, 1998), similar to Desc47 which also remains soluble upon boiling and accumulates in response to water loss.

Desc47's heat stability strongly suggests that it is also an extremely hydrophilic polypeptide, which probably shares biochemical structures similar to those of COR and LEA proteins which enable them to have high affinity to water. The hypothesis that Desc47 is a COR or LEA-like protein still needs to be verified by future cloning of the gene encoding Desc47.

Heat-stability seems to be a common biochemical property of water-stress-related proteins in plants and entomopathogenic nematodes. This may reflect the ability of these unique proteins to endure harsh environments such as extreme temperatures, pH or ionic strength (Jacobsen & Shaw, 1989).

In addition, Desc47's heat stability might indicate a role in the protection of other proteins or cellular structures during the dehydration process as has been proposed for LEA and COR proteins (Dure, 1993*a*, *b*; Thomashow, 1998). Constitutive expression of *Arabidopsis thaliana* COR15a gene in transgenic plants increased the cryostability of biological membranes (reviewed by Thomashow, 1998). Rice plants transformed with the barley HVA1 gene (encoding a group 3 LEA protein) were shown to be highly resistant to desiccation and salt stress, and the extent of their stress tolerance was correlated with the level of HVA1 protein accumulation (Xu *et al*. 1996). In yeast, expression of the tomato LE25 gene (encoding a group 4 LEA protein) also improved resistance to high salinity and freezing (Imai *et al*. 1996). Nevertheless, the biological function of the LEA and COR proteins in the protection mechanisms against desiccation and freezing stress still remains unclear.

Some studies have reported that water-stress-related proteins disappear relatively quickly (within hours) during the rehydration process. For example, the boiling-stable protein BspA, which accumulates in cultured shoots of *Populus tremula* L. in response to water loss, nearly disappear after 4 h of rehydration (Pelah *et al*. 1995). LEA proteins in soybean seeds degrade and disappear after 24 h of imbibition (Blackman *et al*. 1991). In contrast, the novel LEA protein *Pvlea*-18 remains elevated in bean seedling cotyledons, even after 6 days of imbibition (Colmenero-Flores *et al*. 1999). A similar result was found in our study: Desc47 retained its high content in post-desiccated IJs, even after 72 h of rehydration. We suggest that the low turnover of Desc47 implies its role in cellular mechanisms of stress-damage protection and repair. A similar phenomenon has also been described in crayfish, reviewed by Sanders (1993). In that study, heat-shock protein 70 was induced after neural injury and remained elevated for weeks in the injured tissue. The duration and high accumulation of that stress protein was suggested to indicate its role in the crustacean's ability to protect neural tissue and recover from injuries of the central nervous system.

We show that the appearance of Desc47 is accompanied by accumulation of the soluble sugar trehalose. Trehalose is known to be accumulated to high concentrations by organisms that naturally survive dehydration, with specific properties that protect membranes and proteins against desiccation in a model system (Crowe & Crowe, 1992). Trehalose is suggested to stabilize dry membranes by replacing the water molecules around the head groups of the phospholipids (Crowe, Crowe & Chapman, 1984). In yeast, trehalose also promotes survival under conditions of extreme heat, by enabling proteins to retain their native conformation at elevated temperatures and by suppressing the aggregation of denatured proteins (Singer & Lindquist, 1998). Holmstrom *et al*. (1996) demonstrated a significant improvement in drought-tolerance of tobacco seedlings transformed with the trehalose-6-phosphatase synthase subunit gene due to the consequent synthesis of trehalose. Guo *et al*. (2000) demonstrated that human primary fibroblasts expressing trehalose could be maintained in the dry state for up to 5 days.

Blackman, Obendorf & Leopold (1992) concluded that the acquistion of desiccation tolerance in seeds has two prerequisites: synthesis of the heat-stable LEA proteins and cytoplasmic vitrification caused by the accumulation of soluble sugars. Cytoplasmic vitrification of the anhydrobiotic organism's cells may minimize desiccation-stress damage to cellular structures (free-radical oxidation, lipid phase transition and cytoplasmic crystallization) and can protect the physiological integrity of the organism during dehydration and rehydration (Sun & Leopold, 1997). Walters, Ried & Walker-Simmons (1997) demonstrated that heat-stable proteins from mature wheat embryos contain high levels of sugars which appear to be tightly associated with the proteins. The protein-sugar mixture was shown to have a high absorption capacity for water and lower desorption rates under dry conditions. It is suggested that these proteins function as hydration buffers, to control drying so that cells can maintain critical water potentials for the required amounts of time.

In summary, we have identified for the first time in nematodes, a novel water-stress-related protein which exhibits a biochemical property (heat stability) similar to water-stress-related proteins from plants. Acquisition of desiccation tolerance in the entomopathogenic nematode *S. feltiae* has been shown in our study to be correlated to the accumulation of Desc47, and to elevated levels of trehalose. We are presently attempting to clone the gene encoding Desc47 protein. Results will enable us to test our hypothesis that nematodes and plants share homologous genes which are responsive to water loss.

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REFERENCES

BAKER, J., STEELE, C. & DURE, L. (1988). Sequence and characterization of 6 Lea proteins and their genes from cotton. *Plant Molecular Biology* **11**, 277–291.

BARRETT, J. (1991). Anhydrobiotic nematodes. *Agricultural Zoology Review* **4**, 161–176.

BLACKMAN, S. A., WETTLAUFER, S. H., OBENDORF, R. L. & LEOPOLD, A. C. (1991). Maturation proteins associated with desiccation tolerance in soybean. *Plant Physiology* **96**, 868–874.

BLACKMAN, S. A., OBENDORF, R. L. & LEOPOLD, C. (1992). Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. *Plant Physiology* **100**, 255–230.

BOEHRINGER-MANNHEIM (1986). *Methods of Biochemical Analysis and Food Analysis Using Test Combinations*. Boehringer–Mannheim Biochemical GmbH, Mannheim, FRG.

CLOSE, T. J. (1996). Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiological Plantarum* **97**, 795–803.

CLOSE, T. J. (1997). Dehydrins: a commonalty in the response of plants to dehydration and low temperature. *Physiological Plantarum* **100**, 291–296.

CLOSE, T. J., KORTT, A. A. & CHANDLER, P. M. (1989). A c-DNA based comparison of dehydration induced proteins (dehydrins) in barley and corn. *Plant Molecular Biology* **13**, 95–108.

COLMENERO-FLORES, J. M., MORENO, L. P., SMITH, C. E. & COVARRUBIAS, A. A. (1999). Pvlea-18, a member of a new late-embryogenesis-abundant protein family that accumulates during water stress and in growing regions of well-irrigated bean seedlings. *Plant Physiology* **120**, 93–103.

CROWE, J. H. & CROWE, L. M. (1992). Membrane integrity in anhydrobiotic organisms: towards a mechanism for stabilizing dry cells. In *Water and Life* (ed. Somerso, G. N., Osmond, C. B. & Bolis, C. L.), pp. 87–103. Springer-Verlag, Berlin, Germany.

CROWE, J. H., CROWE, L. M. & CHAPMAN, D. (1984). Preservation of membranes in anhydrobiotic organisms: the role of trehalose. *Science* **223**, 701–703.

DURE, L. III (1993*a*). A repeating 11-mer amino acid motif and plant desiccation. *Plant Journal* **3**, 363–369

DURE, L. III (1993*b*). Structural motifs in LEA proteins. In *Plant Response to Cellular Dehydration During Environmental Stress. Current Topics in Plant Physiology* (ed. Close, T. J. & Bray, E. A.), pp. 91–103. American Society of Plant Physiologists, Rockville, MD, USA.

DUTKY, S. R., THOMPSON, J. V. & CANTWELL, G. E. (1964). A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology* **5**, 417–422.

ELLENBY, C. (1968). Determination of the water content of nematode worms by interference microscopy. *Experientia* **24**, 84–85.

GEORGIS, R. & MANWEILER, S. A. (1994). Entomopathogenic nematodes: a developing biocontrol technology. *Agricultural Zoology Reviews* **6**, 63–94.

GHOSH, S., GEPSTEIN, S., HEIKKILA, J. J. & DUMBROFF, E. B. (1988). Use of scanning densitometer or an Elisa plate reader for measurements of nanogram amounts of protein in crude extracts from biological tissues. *Analytical Biochemistry* **169**, 227–233.

GUO, N., PUHLEV, I., BROWN, D. R., MANSBRIDGE, J. & LEVINE, F. (2000). Trehalose expression confers desiccation tolerance on human cells. *Nature Biotechnology* **18**, 168–171.

HILL, R. D., HLADUN, L. S., SCHERER, S. & POTTS, M. (1994). Water stress proteins of *Nostoc commune* (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associated with 1,4-β-D-Xylanxylanohydrolase activity. *Journal of Biological Chemistry* **269**, 7726–7734.

HOLMSTROM, K. O., MANTYLA, E., WELIN, B., MANDAL, A., PALVA, E. T., TUNNELA, O. E. & LONDESBOROUGH, J. (1996). Drought tolerance in tobacco. *Nature, London* **379**, 683–684.

IMAI, R., CHANG, L., OHTA, A., BRAY, E. A. & TAKAGI, M. (1996). A lea-class gene of tomato confers salt and freezing tolerance when over-expressed in *Saccharomyces cervisiae*. *Gene* **170**, 243–248.

JACOBSEN, J. V. & SHAW, D. C. (1989). Heat-stable proteins and abscisic acid action in Barley aleurone cells. *Plant Physiology* **91**, 1520–1526.

KAYA, H. K. & GAUGLER, R. (1993). Entomopathogenic nematodes. *Annual Review Entomology* **38**, 181–206.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the folin reagent. *Journal of Biological Chemistry* **193**, 265–275.

MATSUDAIRA, P. (1987). Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membrane. *Journal of Biological Chemistry* **262**, 10035–10038.

NEVEN, L. G., HASKELL, D. W., HOFIG, A., LI, Q. & GUY, C. L. (1993). Characterization of a spinach gene responsive to low temperature and water stress. *Plant Molecular Biology* **21**, 291–305.

PELAH, D., SHOSEYOV, O. & ALTMAN, A. (1995). Characterization of BspA, a major boiling-stable, water-stress-responsive protein in aspen (*Populus tremula*). *Tree Physiology* **15**, 673–678.

PERRY, R. N. (1977). A reassessment of the variation in the water content of the larvae of *Nematodirus battus* during the hatching process. *Parasitology* **74**, 133–137.

POINAR, G. O. Jr. (1990). Biology and taxonomy of Steinernematidae and Heterorhabditidae. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. K.), pp. 23–62. CRC Press, Boca Raton, FL, USA.

SALOMON, R. (1989). Partial cleavage of sweet potato feathery mottle virus coat protein subunit by an enzyme in extracts of infected symptomless leaves. *Journal of General Virology* **70**, 1943–1949.

SANDERS, B. M. (1993). Stress proteins in aquatic organisms: an environmental perspective. *Critical Reviews in Toxicology* **23**, 49–75.

SCHERER, S. & POTTS, M. (1989). Novel water stress protein from desiccation-tolerant cyanobacterium. *Journal of Biological Chemistry* **264**, 12546–12553.

SINGER, M. A. & LINDQUIST, S. (1998). Thermotolerance in *Saccharomyces cerevisiae* the Yin and Yang of trehalose. *Trends in Biotechnology* **16**, 460–468.

SOLOMON, A., PAPERNA, I. & GLAZER, I. (1999). Desiccation survival of the entomopathogenic nematode *Steinernema feltiae*: induction of anhydrobiosis. *Nematology* **1**, 61–68.

SUN, W. Q. & LEOPOLD, A. C. (1997). Cytoplasmic vitrification and survival of anhydrobiotic organisms. *Comparative Biochemistry and Physiology* **117**, 327–333.

THOMASHOW, M. F. (1998). Role of cold-responsive genes in plant freezing tolerance. *Plant Physiology* **118**, 1–7.

WALTERS, C., RIED, J. L. & WALKER-SIMMONS, M. K. (1997). Heat-soluble proteins extracted from wheat embryos have tightly bound sugars and unusual hydration properties. *Seed Science Research* **7**, 125–134.

WINSTON, P. W. & BATES, D. H. (1960). Saturated salt solutions for the control of humidity in biological research. *Ecology* **41**, 232–237.

WOMERSLEY, C. A. (1981). Biochemical and physiological aspects of anhydrobiosis. *Comparative Biochemistry and Physiology* **70**, 669–678.

WOMERSLEY, C. A. (1987). Reevaluation of strategies employed by nematode anhydrobiotes in relation to their natural environment. In *Vistas on Nematology* (ed. Veech, J. A. & Dickson, D. W.), pp. 165–173. Society of Nematologists, Hyattsville, MD, USA.

WOMERSLEY, C. A. (1990). Dehydration survival and anhydrobiotic potential. In *Entomopathogenic Nematodes in Biological Control* (ed. Gaugler, R. & Kaya, H. K.), pp. 117–137. CRC Press, Boca Raton, FL, USA.

XU, D., DUAN, X., WANG, B., HONG, B., TUAN-HUA, D. H. & WU, R. (1996). Expression of a late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiology* **110**, 249–257.