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Expression and purification of a small heat shock protein from the plant pathogen *Xylella fastidiosa*

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Abstract

The small heat shock proteins (smHSPs) belong to a family of proteins that function as molecular chaperones by preventing protein aggregation and are also known to contain a conserved region termed α-crystallin domain. Here, we report the expression, purification, and partial characterization of a novel smHSP (HSP17.9) from the phytopathogen *Xylella fastidiosa*, causal agent of the citrus variegated chlorosis (CVC). The gene was cloned into a pET32-Xa/LIC vector to over-express the protein coupled with fusion tags in *Escherichia coli* BL21(DE3). The expressed HSP17.9 was purified by immobilized metal affinity chromatography (IMAC) and had its identity determined by mass spectrometry (MALDI-TOF). The correct folding of the purified recombinant protein was verified by circular dichroism spectroscopy. Finally, the HSP17.9 protein also proved to efficiently prevent induced aggregation of insulin, strongly indicating a chaperone-like activity.

Keywords: Small heat shock protein; *Xylella fastidiosa* smHSP; Expression and purification

The heat shock proteins belong to the class of molecular chaperones defined as "cellular proteins which mediate the correct folding of other polypeptides, and in some cases their assembly into oligomeric structures, but which are not components of the final functional structures" [1]. This implies that the main function of chaperones is to transiently interact with proteins avoiding aggregations that are deleterious to the cell. Heat shock proteins are produced by the cells under stress conditions, such as high temperatures, but are also developmentally regulated under physiological condition [2]. They are divided into families according to their size and amino-acid sequence. The small heat shock proteins (smHSPs) belong to a structurally divergent group within the chaperone superfamily, with molecular masses ranging from 12 to 43 kDa [3]. They are also known to contain an evolutionarily conserved region termed α-crystallin domain, located toward the C-terminus of the protein monomer that proved to be important for oligomer formation, thermotolerance, and chaperone activity [1,2]. Circular dichroism analysis of smHSPs indicates that their secondary structures are composed mainly of β-sheets and the C-terminus extensions are likely to be highly flexible [4].

A common feature of small heat shock proteins is their tendency to assemble into large oligomeric complexes, ranging from 145 kDa to 10 MDa [1,5]. Recent studies have demonstrated that oligomers exhibit a dynamic equilibrium with constituent subunits, which is important but not sufficient itself for the chaperone activity [2,6]. The simplest quaternary structure presented in the literature is the 145 kDa trimers of trimers formed by *Mycobacterium tuberculosis* HSP16.3 [5]. Despite the

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interesting properties of the smHSPs and their role in the mechanisms of protein folding, there is little literature on protein structural information, especially three-dimensional structures. One of the few and best-characterized smHSP structures is the *Methanococcus jannaschii* HSP16.5 that has been crystallized revealing a monomer containing a composite β-sandwich structure. It also revealed a highly ordered oligomeric structure composed of 24 monomers forming a hollow spherical complex of octahedral symmetry [7].

In this work, we present the expression, purification, and partial characterization of a novel smHSP from the phytopathogen *Xylella fastidiosa*. The *X. fastidiosa* is a xylem-limited bacterium responsible for a variety of economically important plant diseases, including the citrus variegated chlorosis (CVC) that causes tremendous damage to the citrus cultures in southern Brazil [8–11]. The work presented here aims to contribute to increasing the knowledge of the biological role of smHSPs, presenting a method for the production of high amounts of relatively pure protein that are needed to determine the three-dimensional structure. It also aims at adding new information about proteins that may be related to *X. fastidiosa* pathogenesis, necessary for new approaches towards CVC combat.

Materials and methods

Materials

The oligonucleotide primers were synthesized at Invitrogen Life Technologies (São Paulo, Brazil). The pET32-Xa/LIC vector, the BL21(DE3) strain, protease factor Xa, and anti-His-tag anti-body were obtained from Novagen (Madison, WI). The Ni–NTA affinity resin was obtained from Qiagen (Hilden, Germany). The Coomassie blue reagent for total soluble protein determination was purchased from Bio-Rad (Hercules, CA). The molecular mass marker (LMW) and the HR5/5 (50×5 mm) chromatography column were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). The protease inhibitor phenylmethylsulfonyl fluoride (PMSF), 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), α-cyano-4-hydroxycinnamic acid (CHCA), lysozyme, bovine serum albumin (BSA), and the enzyme trypsin were purchased from Sigma Chemical (St. Louis, MO). The high purity bovine insulin (97% purity) was obtained from Biobrás S.A., (Montes Claros, Brazil). All other chemicals used were of at least reagent grade.

Methods

Expression vector construction

The target ORF (XF2234) from *X. fastidiosa* was amplified by polymerase chain reaction (PCR) using genomic DNA as the template and cloned into the pET32-Xa/LIC expression vector. A pair of PCR primers, 5′-GGTATTGAGGGTCGCATGAACGTTG TTCG-3′ (sense) and 5′-AGAGGAGAGTTAGAGCC TCACATTGACTG-3′ (antisense), was designed to generate products with vector cohesive overhangs. The amplification protocol consisted of a 3 min denaturation at 94 °C followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and 30 s, and extension at 72 °C for 2 min. The blunt PCR products were purified and treated with T4 DNA polymerase in the presence of the dGTP and DTT to generate the specific vector cohesive overhangs. The insert cloning in the linearized vector was carried out according to the vector manufacturer's protocol [6]. The pET32-Xa/LIC vector is designed for the expression of the recombinant protein fused to the 109 amino acid thioredoxin (11.7 kDa), a 6 amino acid His-tag, and 15 amino acid S-tag sequences upstream of the cloning site. The fusion tags together have a molecular mass of 17.6 kDa, a theoretical p*I* of 5.95, and they can be removed from the recombinant target protein by protease cleavage using factor Xa.

Competent *Escherichia coli* DH5α cells were transformed with recombinant plasmids by using a slightly different method of the standard polyethylene glycol (PEG) method [12]. The standard method describes a heat shock step when cells are kept at 42 °C for 30 s. The high temperature step was substituted by letting the cells stand at room temperature for 10 min. Cells were cultured overnight at 37 °C in Luria–Bertani broth (LB) plates containing 50 μg/mL ampicillin. The colonies were individually stored at −70 °C in a permanent 2YT + HMFM broth, which contained 16 g tryptone, 10 g yeast extract, 5 g NaCl, 0.076 g $MgSO_4 \cdot 7H_2O$, 0.45 g dehydrated sodium citrate, 0.90 g $(NH_4)_2SO_4$, 1.8 g KH_2PO_4, 4.7 g K_2HPO_4, and 44 mL glycerol, per liter. The clones were checked by PCR and their sequences were verified by nucleotide sequencing. *E. coli* BL21(DE3) cells were transformed with positive recombinant plasmid using the PEG method and used for protein expression.

smHSP over-expression and purification

Escherichia coli BL21(DE3) cells transformed by recombinant plasmids were cultured in 10 mL LB broth added to 50 μg/mL ampicillin and grown overnight at 37 °C and 300 rpm. This pre-inoculum was then transferred to 1.0 liter LB broth containing ampicillin at the same concentration. The culture was grown at 37 °C and 300 rpm until OD_{600} of 0.8 when cells were induced with 1 mM IPTG for an additional 2 h. The cells were harvested by centrifugation at 2600*g* and 4 °C for 10 min. The pellet containing the bacteria was suspended in 44 mL of 50 mM Tris–HCl buffer, pH 7.5, containing

300 mM NaCl (adsorption buffer). The protease inhibitor PMSF and lysozyme were added to final concentrations of 1.0 mM and 1.0 mg/mL, respectively. The suspension was incubated for 30 min at 4 °C and sonicated 8 times for 15 s at 70% of the maximum power in a Sonifier Misonix (Microson Ultrasonic Cell Disruptor XL). The lysed material was clarified by centrifugation at 27,500*g* for 15 min at 4 °C and the supernatant was collected to confirm recombinant protein expression by SDS–PAGE.

Small-scale purification of the target protein was done by loading 6 mL of the clarified supernatant onto an HR5/5 column, packed with 1.0 mL nickel–nitrilotriacetic acid (Ni–NTA) resin, using an AKTA-FPLC system. The resin was washed with 10 CV of adsorption buffer containing 1.0 M NaCl. The adsorbed proteins were eluted from the column using an imidazole gradient (from 0 to 200 mM in 10 CV) in adsorption buffer. Fractions containing 1.0 CV of volume were collected and a flow rate of 0.75 mL/min was used during all the chromatographic steps. Fractions were assayed for total protein concentration and analyzed by SDS–PAGE. The recombinant HSP17.9 was separated from the fusion tags by factor Xa proteolysis (0.1 U/μL per 10 μg of recombinant protein). The hydrolysis was performed at 25 °C for 16 h according to the protease supplier's instructions. The protease was inactivated by PMSF at 1 mM and the proteolysis products were analyzed by SDS–PAGE. The sample was dialyzed against adsorption buffer containing 10 mM imidazole to prepare for the next purification step.

The final purification step was performed using the same FPLC system and column/resin mentioned above. The sample containing the HSP17.9 and the cleaved fusion protein was loaded into the column equilibrated with the same dialysis buffer. The flowthrough proteins (HSP17.9) were collected and washed out with 10 CV of the same buffer. Bound proteins were eluted with equilibration buffer containing 200 mM imidazole. The flowrate used was 0.75 mL/min. The fractions containing 1 CV of volume were collected and analyzed by SDS–PAGE and Western blot, and had their total soluble protein concentration determined. Large-scale purification was performed by scaling up the same protocol described above, except for the column/resin used. In this case, clarified extract from 5.0 liters of bacterial culture was loaded into an HR10/10 column packed with 8.0 mL of agarose–IDA–Ni^{2+} resin. All chromatographic steps were carried out at 2.0 mL/min.

Analytical assays

The total soluble protein concentration was assayed according to the method presented by Bradford [13], using bovine serum albumin as the protein standard. More accurate estimations for purified HSP17.9 were made based on absorbance at 280 nm, using a calculated extinction coefficient of 0.852 g/L/cm, based on the method described by Pace and Schmidt [14]. Protein purity during the purification step was monitored by SDS–PAGE performed using a 4% stacking gel and a 12% separation gel according to Laemmli [15] and stained using the Coomassie brilliant blue R-250 staining technique. Native gel electrophoresis was carried out using the same protocol described above, subtracting the detergent sodium dodecyl sulfate (SDS) from all the buffers used. For Western blot, protein samples from the second chromatographic step were run on 12% gel SDS–PAGE and transferred to nitrocellulose membranes (Hybond-N, Amersham, USA). Detection was carried out using rabbit anti-His-tag antibody and a conjugated goat anti-rabbit igG alkaline phosphatase (Sigma, St. Louis, MO) followed by chemiluminescent detection with CSPD (Tropix, USA).

Mass spectrometry

The recombinant HSP17.9 mass spectrometric analysis was done in a matrix-assisted laser-desorption time-of-flight (MALDI-TOF) M@LDI-LR mass spectrometer (Micromass, USA). The trypsin-digested protein and intact protein were analyzed in the spectrometer using reflecting and linear modes, respectively, according to the equipment manufacturer's instructions. The matrixes used for trypsin-digested (peptides) and intact protein samples were CHCA and sinapinic acid, respectively. The masses of monoisotopic peaks with relative intensity higher than 5% of the most intense peak in the spectrum were used for comparison to a theoretical digestion of the protein by trypsin. This was carried out using the MS-Digest program (www.http://prospector.ucsf.edu).

Circular dichroism spectroscopy

The circular dichroism (CD) spectroscopy of the fully purified protein was studied to assess the secondary structural integrity. The CD spectra were generated using a 1 mm pathlength cuvette containing 200 μL HSP17.9 protein sample at 0.1 mg/mL in 5 mM Tris–HCl buffer, pH 7.5, at 20 °C. The assays were carried out in a Jasco 810 spectropolarimeter (Japan Spectroscopic, Tokyo, Japan). The spectrum was presented as an average of four scans recorded from 190 to 250 nm, at a rate of 20 nm/min.

Chaperone-like activities study

The inhibition of the aggregation of bovine insulin (B chain) by HSP17.9 was studied according to the method presented by Horwitz et al. [16]. The method is based on the reduction of insulin disulfide bonds by dithiothreitol

and aggregation of insulin B chain followed by the increase of the turbidity from light scattering at 360 nm in a Hitachi U-3000 spectrophotometer. All experiments were carried out at 37 °C.

Results and discussion

Expression and purification of HSP17.9

The *X. fastidiosa* Genome Program generated a large amount of gene sequences from this important plant pathogen [11]. One of these genes encodes a 17.9 kDa protein similar to a family of small heat shock proteins. The authors in the literature suggest that the minimal functional unit of these kinds of proteins consists of a core region of about 85 amino acids called the α-crystallin domain. Fig. 1 presents the sequence alignment of the *X. fastidiosa* protein, called HSP17.9, with three other proteins that may belong to the same family. The strongest similarity (26%) was found with the SP21 protein (Q06823) from *Stigmatella aurantiaca*. The alignment also compares the HSP17.9 with the *M. jannaschii* HSP16.5 (Q57733) and human α-crystallin (B53814). As expected, the highest number of hits was found in the α-crystallin domain, the highly conserved domain of smHSPs.

In our work, the ORF (XF2234) encoding the HSP17.9 protein was successfully cloned in the pET32 Xa/LIC expression vector and no difficulty was found to transform the *E. coli* BL21(DE3) strain for expression studies. Dose dependence and time course studies of the induction of the recombinant protein expression, analyzed by SDS–PAGE, led to an IPTG concentration of 1 mM and induction time of 2 h at 37 °C. The expression of the fusion protein containing the HSP17.9 was considered high: 9–10 mg of soluble protein/liter of bacterial broth. High amounts of relatively pure recombinant protein containing the His-tag were recovered and purified from the lysed extract by using immobilized metal affinity chromatography (Fig. 2, lanes 2–4). The reduction of non-specific interactions between extract proteins and the matrix was done washing the resin with high salt concentration and low imidazole concentration buffers. The elution of the recombinant protein was performed using a gradient of imidazole concentration, a procedure that considerably improved the final protein purity. Elution fractions containing the recombinant protein with a level of purity higher than 90% (analyzed by optic densitometry) were separated and combined as

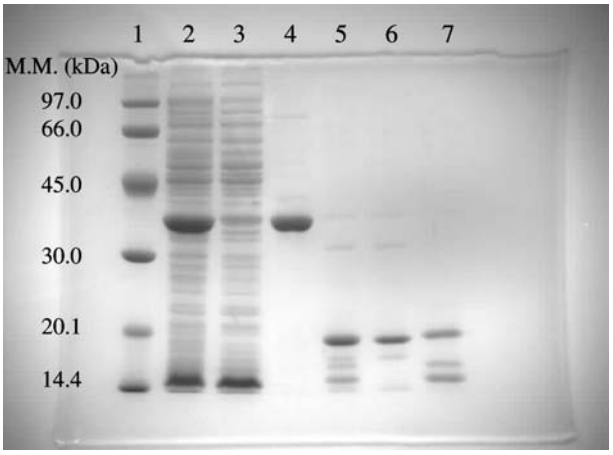

Fig. 2. SDS-PAGE of the fractions collected during the purification steps of the recombinant HSP17.9. Legend: lane 1—molecular mass markers; lane 2—extract from bacterial lysis loaded into the Ni–NTA column, 11.0 μg; lane 3—flowthrough extract, 7.0 μg; lane 4—fraction collected during the elution step at imidazole concentration of 200 mM, containing the recombinant protein (HSP17.9 + fusion tags), 4.0 μg; and lane 5—proteins resulted from factor Xa cleavage step for HSP17.9 separation from fusion tags, 3.2 μg. This fraction was loaded into the Ni–NTA column to promote final purification of the HSP17.9. Lane 6—flowthrough fraction of the final purification step containing the HSP17.9, 2.0 μg. Lane 7—proteins eluted at 200 mM imidazole concentration containing the fusion tags and impurities resulted from non-specific factor Xa cleavage, 2.3 μg. Separation gel of 12% acrylamide concentration.

```
HSP17.9             1 ---------MNVVRYT------PWPGQAALQNEIKQVFDRFFEHNGDTDES---AVVTAQ
SP21                1 ------MADLSVRRGTGSTPQRTREWDPFQQMQELMNWDPFELANHPWFAN---RQGPPA
HSP16.5             1 ----------MFGRDP-------FDSLFERMFKEFFATPMTGTTMIQSSTGI---QISGKG
alfa-crystallin     1 MEIPVPVQPSWLRRASAPLPGLSAPGRLFDQRFGEGLLEAELAALCPTTLAPYYLRAPSV

HSP17.9            43 WVPRVDIKEEPNQFVLYADLPGIDPADIEVQMDKGILSIKGERKTPSSSQTEHFSRIERR
SP21               52 FVPAFEVRETKEAYIFKADLPGVDEKDIEVTLTGDRVSVSGKRERPKREESERFYAYERT
HSP16.5            42 FMP-ISIIEGDQHIKVIAWLPGVNKEDILNAVGDTLEIRAKRSPLMITESERIIYSEIP
alfa-crystallin    61 ALPVAQVPTDPGHFSVLLDVKHFSPEEIAVKVVGEHVEVHARHEERPDEHG--------F

HSP17.9           103 YG-SFHRRFALPDSADADGITASGSH-GVLSIFIPKRAATTPRRIQVGNAVSNDVALQSM
SP21              112 FG-SFSRAFTLPEGVDGDNVRADLKN-GVLTLTLPKRPEVQPKRIQVASSGTEQKEHIKA
HSP16.5           101 EEEEIYRTIKLPATVKEENASAKFEN-GVLSVILPKAESSIKKGINIE------------
alfa-crystallin   113 VAREFHRRYRLPPGVDPAAVTSALSPEGVLSIQAAPASAQAPPPAAAK------------

HSP17.9           161 --------------------
SP21              170 YPAPAEPGLAAPLGWPGFS
HSP16.5               --------------------
alfa-crystallin       --------------------
```

Fig. 1. Comparison of the *X. fastidiosa* HSP17.9 with other smHSPs. The multiple sequence alignment of the HSP17.9 with *S. aurantiaca* SP21 (Q06823), *M. jannaschii* HSP16.5 (Q57733), and human alfa-crystallin (B53814) was done using the CLUSTALW program. Letters shaded in black indicate amino acids that are identical in at least two of the smHSPs. Conserved substitutions are shaded in gray.

a pool for further purification steps. Approximately 6 mg of purified fusion recombinant protein/liter of initial bacterial broth was recovered at this step.

To remove the N-terminal fusion tags from the target protein, a cleavage by factor Xa protease was performed. The fusion tags were used so as to improve the recombinant protein solubility, also allowing ease of detection and purification from the extract. The fusion tags were successfully removed from HSP17.9 by cleavage and few non-specific cleavages were noted. The analysis on SDS–PAGE of the proteolysis products is shown in Fig. 2 (lane 5). Since the recombinant HSP17.9 and the fusion tags contain almost identical molecular masses (17.9 and 17.6 kDa, respectively), they are seen on the gel as a unique protein band. The cleaved recombinant HSP17.9 remained stable in the solution, however, some tendency towards aggregation and precipitation was noticed when the protein was dialyzed against water at a low temperature (4 °C).

A final chromatographic step on the IMAC resin was performed to separate the fragment containing the tags and the target protein. In this case, the purified recombinant HSP17.9 was collected in the flowthrough fractions (Fig. 2, lane 6). Few amounts of non-cleaved protein were also separated from the target protein during this step. Since both proteins contain similar molecular masses, merged as a single band in SDS–PAGE, the fractions collected during the chromatography were analyzed by Western blot using anti-His-tag antibody. The result indicated that the separation was achieved, since the flowthrough fractions were virtually free from proteins containing His-tag (result not shown). The final amount of purified recombinant HSP17.9 obtained was approximately 1.6 mg/liter of initial bacterial broth. More detailed data on the purification of the HSP17.9 are presented in Table 1.

Mass spectrometry and oligomerization analysis

The purified recombinant HSP17.9 was analyzed by mass spectrometry to verify the protein molecular mass and identity. The analysis resulted in a molecular mass of 17,729 Da, which is a value close to the theoretical molecular mass calculated from the primary amino-acid sequence: 17,858 Da. The peptide profile found for the mass spectroscopy analysis of the digested HSP17.9 (“fingerprint”) was compared to a theoretical digestion of the protein. Five different peptides were found the molecular masses of which matched (difference lower than 1.0 Da) those of the expected peptides, confirming the protein identity (data not shown).

The HSP17.9 tendency to assemble into oligomeric complexes was also detected by native gel electrophoresis and gel filtration chromatography analysis. Despite the results for the determination of the exact molecular mass of the protein quaternary structure still not being conclusive, maybe due to a natural instability of such complexes, there is no doubt that the protein tends to form large oligomeric complexes in solution. Recent studies have suggested that the assemblies of smHSPs high level structures are dynamic and shaped by the frequent exchange of subunits [1]. The studies have also suggested that these structures are important but not sufficient for the chaperone-like activity [2,6].

Circular dichroism spectroscopy analysis

To investigate the structural integrity of the purified HSP17.9, the protein was analyzed by CD spectroscopy. The CD spectrum, which resulted, is shown in Fig. 3 and indicated that the recombinant HSP17.9 contains a substantial amount of secondary structure. The protein presented a predominant signal of β-strands, with a maximum in ellipticity at 197 nm and a minimum at 213 nm. This result is coherent with the secondary-structure predictions and data presented in the literature for other smHSP structures, all of them making up a majority of β-strands. One of the best structurally characterized smHSPs in the literature, the *M. jannaschii* HSP16.5, was found to have its monomer formed by nine β-strands, two short 3_{10}-helices, and one short β-strand [7]. Finally, our results indicated that the

Table 1
Purification of recombinant HSP17.9 from *E. coli* extract

Step	Volume (mL)	Protein (mg)	HSP17.9 (mg)	HSP 17.9 yield (%)	HSP 17.9 purity (%)	Purification factor
Extraction	44	79	9.0	100	11	1
First IMAC[a]						
Flowthrough	84	68	0	0	—	—
Eluate	80	6.7	6.0	67	90	8.2
Second IMAC						
Load[b]	32	5.7	2.3	26	40	3.6
Flowthrough	37	3.5	0	0	—	—
Eluate	10	1.8	1.6	18	89	8.1

[a] Immobilized metal ion chromatography.
[b] This step is preceded by a cleavage step using Factor Xa protease. The cleaved HSP17.9 usually represents about 40% of the resulting total protein in solution.

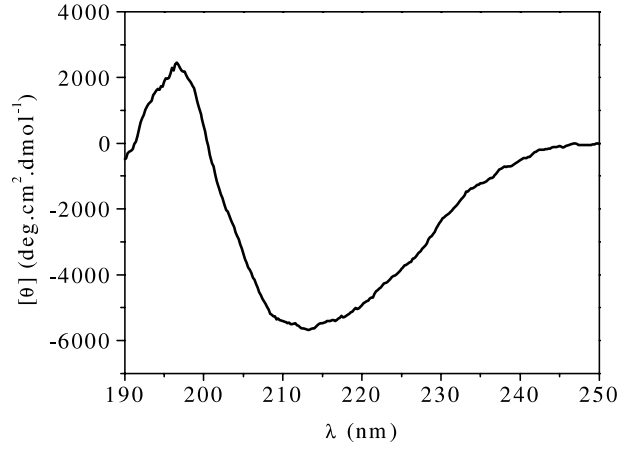

Fig. 3. Circular dichroism spectrum of the purified recombinant HSP17.9. The spectrum was recorded at 20 °C and a protein concentration of 0.1 mg/mL in 5 mM tris buffer, pH 7.0. Data were collected using an average of four scans per replicate.

protein is quite stable at room temperature and remained folded throughout the purification process, being suitable for crystallization studies aiming at three dimensional structure determinations.

Chaperone-like activity study

It is well known that some proteins can be unfolded by reducing the interchain disulfide bond. In the case of insulin, the reduction of the disulfide bonds by DTT will lead to the aggregation and precipitation of the B chain while the A chain remains in solution. In this way, the chaperone-like activity of the recombinant HSP17.9 could be indicated by its characteristic of preventing insulin B chain aggregation upon reducing environmental condition. Since the HSP17.9 does not possess any disulfide bond, it is not affected in this condition as insulin. Fig. 4 presents the results found for insulin aggregation in the presence of different mass concentrations of HSP17.9. Significant prevention of insulin aggregation was noted even at low HSP17.9 concentrations (0.02 mg/mL for an insulin concentration of 0.4 mg/mL) strongly indicating a chaperone-like activity. Almost 40% reduction of aggregate formation was detected when a final concentration of 0.08 mg/mL of HSP17.9 was used (20% of the insulin concentration).

Finally, the different effects caused by the presence of HSP17.9, and the control proteins bovine serum albumin (BSA) and lysozyme on the induced aggregation of insulin are illustrated in Fig. 5. The HSP17.9 presented a chaperone-like activity effectively preventing insulin aggregation, whereas BSA interaction seems only to retard it. The lysozyme also aggregated during the assay, causing a shift in the measured light scattering, the opposite effect of HSP17.9.

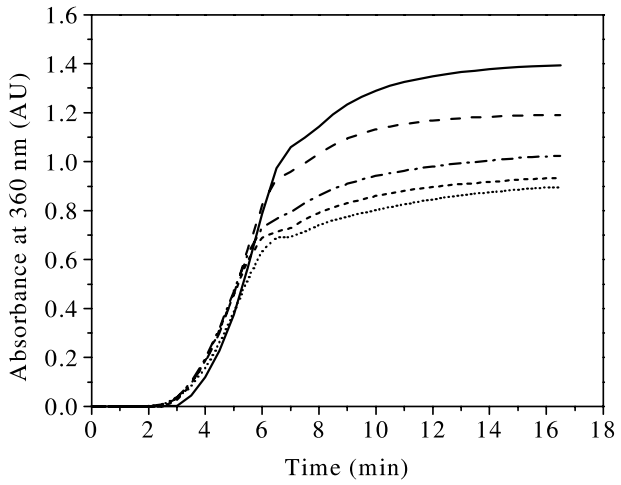

Fig. 4. Profile of insulin aggregation induced by dithiothreitol (DTT) in the presence of different concentrations of the recombinant HSP17.9 protein. Legend: solid line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and absence of HSP17.9; dashed line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.02 mg/mL HSP17.9; dash-dot line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.04 mg/mL HSP17.9; short dash line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.06 mg/mL HSP17.9; short dot line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.08 mg/mL of HSP17.9. Experiments were performed in a 50 mM NaPi buffer, pH 7.5, containing 100 mM NaCl at a temperature of 37 °C.

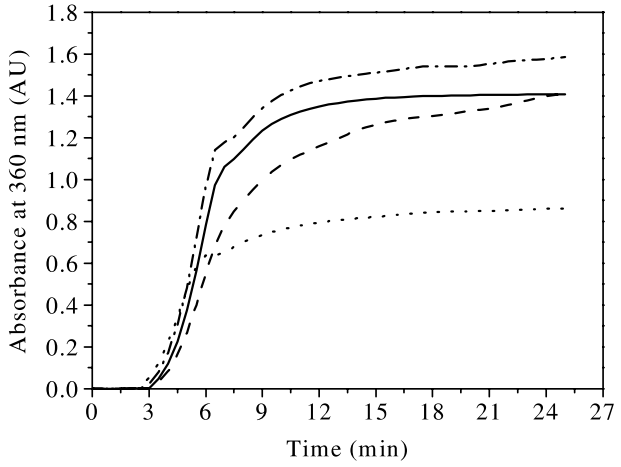

Fig. 5. Profile of insulin aggregation induced by dithiothreitol (DTT) in the presence of recombinant HSP17.9, bovine serum albumin (BSA), and lysozyme. Legend: solid line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT (control); dashed line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.1 mg/mL BSA; dash-dot line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.1 mg/mL lysozyme; and short dot line, 0.4 mg/mL insulin solution in the presence of 20 mM DTT and 0.1 mg/mL HSP17.9. Experiments were performed in a 50 mM NaPi buffer, pH 7.5, containing 100 mM NaCl at a temperature of 37 °C.

Conclusion

This work presented the cloning, expression, purification, and partial characterization of a novel smHSP

protein from the plant pathogen *X. fastidiosa*. The protein was purified using two steps of affinity chromatography on Ni–NTA resin, and its purity and identity were verified by SDS–PAGE, Western blot, and mass spectrometry. As expected for smHSP proteins, the CD analysis of the HSP17.9 indicated a secondary structure composed mainly of β-strands, despite some short α-helices also seeming to be present. The HSP17.9 proved to efficiently avoid chemically induced insulin aggregation, strongly indicating a chaperone-like activity. Finally, this work presents new information about a protein the biological role of which may be related to the *X. fastidiosa* phatogenicity. Crystallization studies of the protein are currently in progress in our laboratory for future three-dimensional structure characterization by X-ray crystallography.

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