



Biochemical characterization of the RNA-hydrolytic activity of a pumpkin 2S albumin

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ABSTRACT

A pumpkin 2S albumin with ribonuclease (RNase) activity was purified from pumpkin seeds (*Cucurbita* sp.) by liquid chromatographic techniques. It manifested potent RNase activity toward baker's yeast RNA and calf liver RNA, and some polyhomoribonucleotides, including poly(A), poly(U) and poly(C) but not poly(G). Moreover, it was able to hydrolyze total RNA of both animal and plant origins. Ions such as Na⁺, Mg²⁺, Ca²⁺, and Zn²⁺ inhibited its RNase activity. Since RNase activity has not been previously reported in 2S albumins, this work may shed further light on the biological importance of this group of proteins.

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1. Introduction

Plant 2S albumins are water-soluble seed storage proteins that share common structural features, including (1) they are typical heterodimers composed of a large subunit and a small subunit which are connected by one or two disulfide bonds, and (2) they contain four helices [1,2]. Although the major function of 2S albumins as seed storage proteins is to provide a source of carbon, nitrogen, and sulfur during germination and the development of seedlings, they also manifest other biological activities including trypsin inhibitory, antimitotic, allergenic, and others [1–3]. To date, no demonstration of ribonuclease (RNase) activity associated with 2S albumins has been reported.

RNA degradation is a determining factor in the control of gene expression. A diversity of RNases are involved in the maturation, turnover and quality control of RNA [4,5]. RNase is a type of enzyme that catalyzes the degradation of RNA into smaller components [4–6]. This important RNA degrading activity of RNases links them with many biological processes and brings them to the foreground of research [4–6].

In this paper we report the purification and identification of a mature 2S albumin from pumpkin seeds. We focused on the description of its RNase activity with presented biochemical characteristics in accordance with some other RNases. The RNA-hydrolytic activity of pumpkin 2S albumin unveils its potential function in RNA regulatory network and may work with potential applications.

2. Materials and methods

2.1. Materials and chemicals

Fresh pumpkin seeds (*Cucurbita* sp.) were acquired from a local vendor and authenticated by Professor Shiu-ying Hu, The Chinese University of Hong Kong. Three RNA preparations, including type X, type III, and type IV, and four polyhomoribonucleotides, poly(A), Poly(G), Poly(C), and Poly(U) were from Sigma. All other chemical reagents were from Sigma unless otherwise stated.

2.2. Purification of pumpkin 2S albumin

Fresh pumpkin seeds were homogenized and centrifuged followed by loading of the supernatant onto an SP Sepharose column in 0.02 M NH₄OAc buffer (pH 4.5). The adsorbed fraction eluted with 1 M NaCl was dialyzed extensively and further fractionated by using an FPLC system equipped with a Mono S 5/50 GL column. Fraction containing the 2S albumin was finally loaded on a Superdex 75 10/300 GL column in 0.02 M NH₄HCO₃ buffer (pH 9.4). The purified pumpkin 2S albumin was dialyzed extensively, freeze-dried, and stored at minus 20 °C for further studies.

2.3. SDS-PAGE and mass spectrometry

SDS-PAGE (15% polyacrylamide gel) and MALDI-TOF mass spectrometry (MS) were carried out for molecular weight determination [7].

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2.4. N-Terminal sequencing and MALDI-TOF MS for protein identification

N-terminal amino acid sequencing was done using the automated HP protein sequencer (Model G1005A Protein Sequencing System) [7]. For further protein identification, purified protein was pretreated with 10 mM dithiothreitol (DTT) at room temperature for 5 h, and submitted to the Applied Biosystems (ABI) 4700 Proteomics Analyzer, MALDI-TOF MS (Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer, USA) for further molecular weight analysis.

2.5. Phylogenetic tree construction and 3-dimensional homology modeling

A phylogenetic tree was constructed using ClustalX 1.83 software and further edited by using the software TreeView [8]. The 3-dimensional homology model for pumpkin 2S albumin was built using the online Phyre web-server [9].

2.6. Assay of RNase activity

The assay of RNase activity was based on spectrophotometric measurement of acid-soluble nucleotides formed from substrate RNA as described elsewhere [10].

2.7. Activity toward polyhomoribonucleotides

The ribonucleolytic activity toward polyhomoribonucleotides was measured on the basis of acid-soluble hydrolysis products released by each protein [10].

2.8. In-solution RNase activity assay toward total RNA

MCF-7 cells and fresh seeds of *Bauhinia variegata* were chosen for preparing total RNA separately [7]. After total RNA extraction [11], about 1 µg of total RNA of one origin was incubated at 37 °C with pumpkin 2S albumin under different conditions. The reaction mixture was then electrophoresed on a 1% non-denaturing agarose gel and stained with ethidium bromide for checking RNA integrity [11].

2.9. Thermal and pH dependence of RNase activity

RNase activity of pumpkin 2S albumin under different temperatures and pH values were determined as mentioned previously [10].

2.10. Effects of cations and DTT on RNase activity

The assay was performed in a reaction volume of 150 µl containing 50 mM sodium phosphate buffer (pH 7.0), 200 µg RNA (type X), and 0.02 µM pumpkin 2S albumin, with one of the following: NaCl, CaCl₂, MgCl₂, ZnCl₂, DTT [12].

For all experimental details, see [Supplementary data](#).

3. Results

3.1. Purification of pumpkin 2S albumin

The crude pumpkin seed extract was first run on an SP Sepharose column. The adsorbed fraction was then resolved into five major adsorbed peaks on a FPLC-Mono S column (Fig. 1A). Fraction corresponding to peak MS-5 was subsequently subjected to a Superdex 75 column and the purified protein (Fig. 1B, fraction SUP-1) was ac-

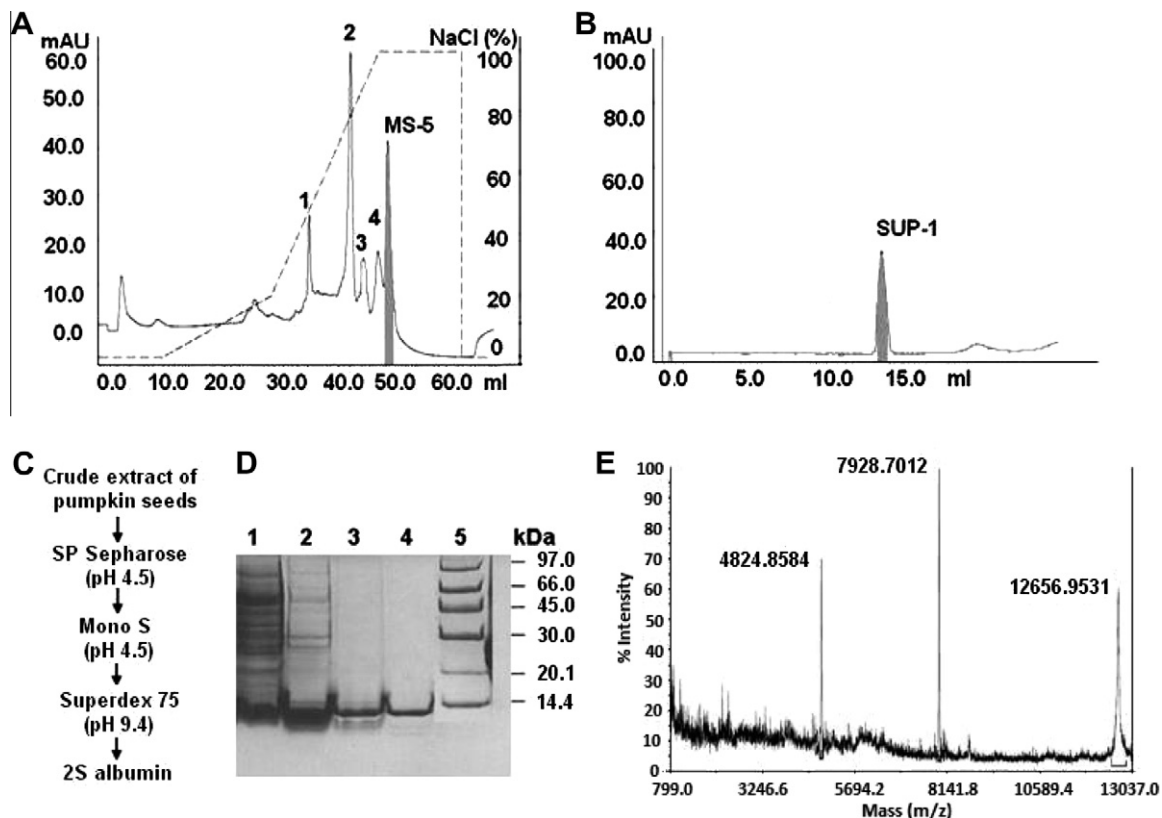


Fig. 1. Purification of pumpkin 2S albumin. (A) Fraction of pumpkin seeds bound on SP-Sepharose was applied to a Mono S 5/50 GL column and five major bound fractions were obtained. (B) Fraction MS-5 from Mono S was subsequently subjected to a Superdex 75 10/300 GL column to yield a major peak SUP-1 representing purified 2S albumin. (C) Schematic representation of the chromatographic steps used for the purification work. (D) SDS-PAGE results. Lane 1, crude extract; lane 2, SP bound fraction; lane 3, fraction MS-5; lane 4, fraction SUP-1; lane 5, protein ladder. (E) MALDI-TOF MS results of purified pumpkin 2S albumin treated by dithiothreitol (DTT) showing a MW of 12 657 Da (peak 3 from left) which was composed of a small chain (peak 1) and a big chain (peak 2) linked by disulfide bonds.

quired. The purification procedure was concluded in Fig. 1C. About 80 mg purified protein was obtained from 285 g fresh pumpkin seeds.

Purity of the isolated protein was established by the appearance of a single band in SDS–PAGE under non-reducing conditions (Fig. 1D, lane 4) corresponding to a molecular weight (MW) around 12.5 kDa. SDS–PAGE under reducing conditions (protein pretreated by DTT) showed that the protein is composed of two disulfide-linked polypeptide chains (Fig. 6F). MALDI-TOF MS of the DTT-treated protein further confirmed this result. As shown in Fig. 1E, it unveiled that the mature protein (12 657 Da, peak 3 from left) is composed of a small chain (peak 1) and a big chain (peak 2) with a MW of about 4825 and 7927 Da, respectively. The observation is in agreement with the presence of two disulfide-linked polypeptides, a small chain (theoretical value about 4826.4 Da) and a big chain (about 7073 Da), in prepro2S albumin [13–15].

3.2. Amino acid sequence and bioinformatics analysis of pumpkin 2S albumin

The sequence of the first 25 and 48 N-terminal amino acids of the small chain and big chain of pumpkin 2S albumin respectively

are shown in Fig. 2. A BLAST search with the acquired sequences of the two chains against the GenBank shows a high homology with the corresponding N-terminal sequence of pumpkin prepro2S albumin [13,14]. MW of the purified protein (12.6 kDa) is the same as that of pumpkin prepro2S albumin in its mature form (theoretical MW, 12.7 kDa) [15]. Based on the results of the purified protein including: (1) its being a heterodimer which contains a large chain and a small chain connected by disulfide bonds (Fig. 1E and Fig. 6F); (2) a pronounced N-terminal sequence homology of both chains of the purified protein to pumpkin prepro2S albumin (Fig. 2); (3) similarity of its MW to pumpkin 2S albumin in its mature form; and (4) the purified protein may contain four helices (Supplementary Fig. 1), it can be concluded that the purified protein from pumpkin seeds should be subsumed as a 2S albumin protein, especially with remarkable homology to the mature form of the previously reported pumpkin prepro2S albumin [13,14].

Sequence alignment between pumpkin 2S albumin and other 2S albumins revealed marked sequence similarity ranging from 78% (with a ribosome-inactivating protein/RIP, luffin S2) to 34% (Fig. 2). Their genetic relationships are summarized in the form of phylogenetic tree (Supplementary Fig. 1C). It is noteworthy that pumpkin 2S albumin was devoid of adenine polynucleotide glyco-

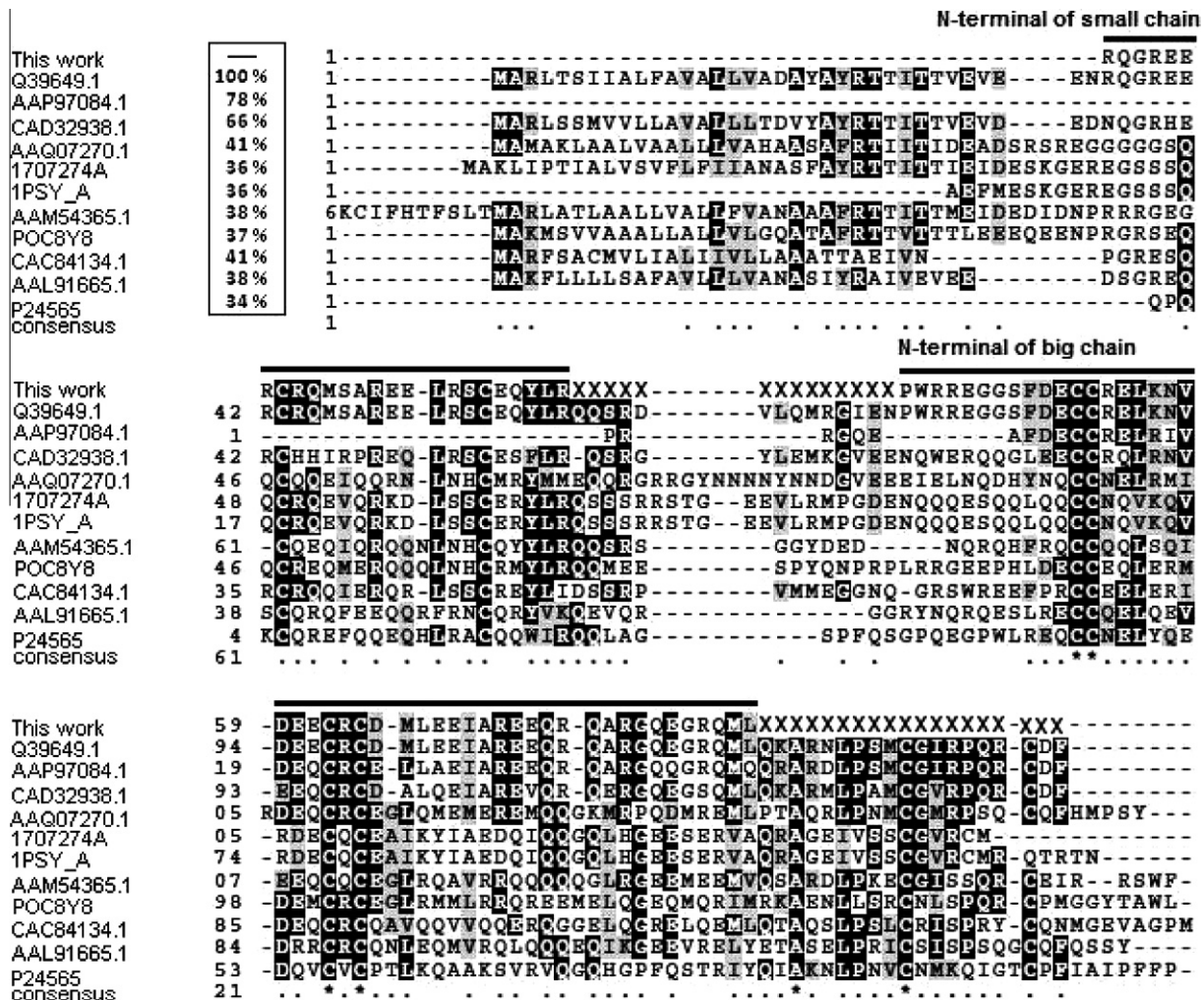


Fig. 2. Amino acid sequence of pumpkin 2S albumin and comparison with related proteins. The rectangle highlights the percentage sequence identity to purified pumpkin 2S albumin (this work). Q39649.1, pumpkin prepro2S albumin (*Cucurbita* sp.); AAP97084.1, ribosome-inactivating protein luffin S2 from *Luffa aegyptiaca*; CAD32938.1, napin from *Momordica charantia*; AAQ07270.1, 2S albumin from *Ficus pumila*; 1707274A, 2S albumin from *Ricinus communis*; 1PSY_A, 2S albumin from *Ricinus communis*; AAM54365.1, 2S albumin from *Juglans nigra*; POC8Y8, 2S sulfur-rich seed storage protein 2 from *Bertholletia excelsa*; CAC84134.1, precursor of thiamin-binding protein from *Sesamum indicum*; AAL91665.1, 2S albumin from *Anacardium occidentale*; P24565, 2S albumins from *Brassica napus*. The asterisk indicates identical amino acids throughout the proteins listed, and residues conserved in >50 % are marked with black dots underneath. N-terminal amino acids of both the small chain and big chain of pumpkin 2S albumin were indicated with bold lines overhead. X, unknown amino acids.

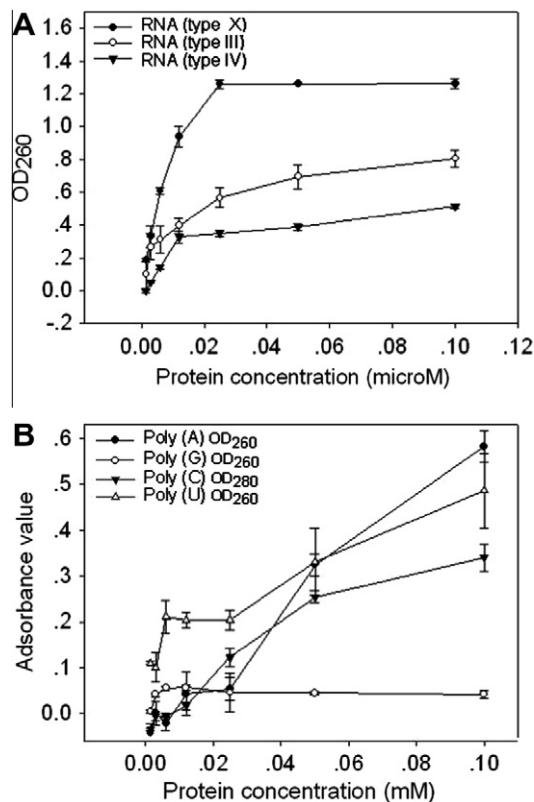


Fig. 3. RNase activity of pumpkin 2S albumin. (A) RNA-hydrolytic activity of pumpkin 2S albumin toward different RNAs (type X, III, and IV) in a dose-dependent manner. (B) Dose dependence of cleavage of polyhomoribonucleotides by pumpkin 2S albumin. In the case of type III and IV RNAs, and poly (U), 10-fold dilution of the supernatant was prepared before the absorbance was determined. Means $\pm$ S.D. of three independent experiments are shown.

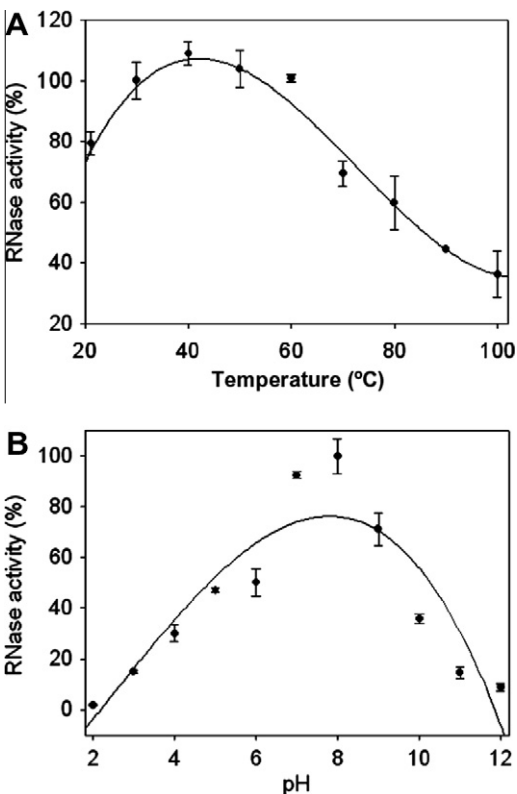


Fig. 5. Effects of temperature and pH on RNase activity of pumpkin 2S albumin. The RNase activity was assayed as a function of temperature (A), and pH (B) using the procedures described in "Section 2". The best fit curves were drawn using the SigmaPlot 10.0 software. Means $\pm$ S.D. of three independent experiments are shown.

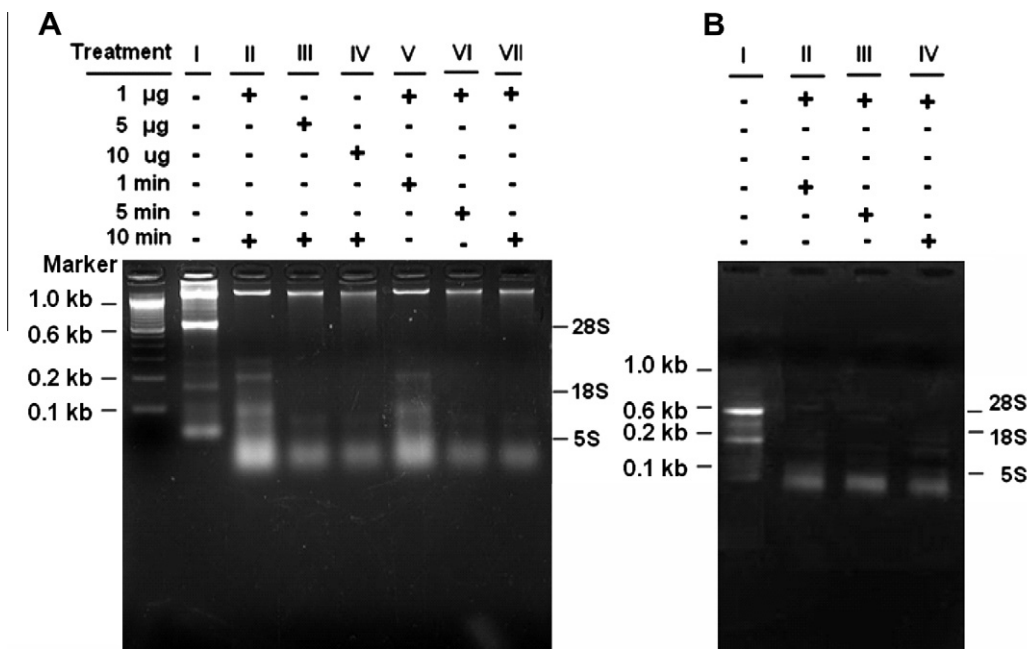


Fig. 4. RNase activity of pumpkin 2S albumin on total RNAs of different origins. (A) About 1 μ g of MCF-7 total RNA was incubated at 37 $^{\circ}$ C with pumpkin 2S albumin of different concentrations from 1 to 10 μ g for 10 min (lanes II–IV) or at the dose of 1 μ g and for the indicated durations from 1 to 10 min (lanes VI–VIII). (B) Its RNase activity toward the total RNA of the plant *Bauhinia variegata* was also determined by incubating with 1 μ g of pumpkin 2S albumin for 1, 5 to 10 min (lanes II, III and IV). Total RNA loaded in each lane was about 1 μ g. Lanes A-I and B-I were negative controls. Experiments were performed 3 times with identical results and one set of representative data is shown.

sylase activity which is an inherent feature of RIP (data not shown) [16,17]. This result excluded pumpkin 2S albumin from being an RIP.

The complete amino acid sequence of pumpkin prepro2S albumin (accession number: Q39649.1) in its mature form was used to predict the 3-D structure of pumpkin 2S albumin reported in this work. By using the on-line Phyre server, a predictive 3-D model of pumpkin 2S albumin in the modes of backbone and ribbon was generated (Supplementary Fig. 1A and B). The crystal structure of 2S albumin RicC3 (GenBank accession number: 1PSY_A) from *Ricinus communis* [18] was used as template (its 3-D structure was presented in Supple-

mentary Fig. 2). Since the template is detected with >30% (36% identity, Fig. 2) sequence identity to pumpkin 2S albumin, most of the alignment should be accurate and the resulting relative positions of structural elements in the model are reliable [9]. This predictive model presents good stereochemistry as further judged by ANOLEA [19]. As shown in Supplementary Fig. 1A and B, mature pumpkin 2S albumin is composed of 4 α -helices (H-I to H-IV, light red) and some loops (blue and white). Analysis by the Symyx Chime browser disclosed that its 3-dimensional structure is stabilized by three disulfide bridges (Supplementary Fig. 1A): Cys8–Cys62 (SS-1), Cys20–Cys51 (SS-2), and Cys52–Cys97 (SS-3).

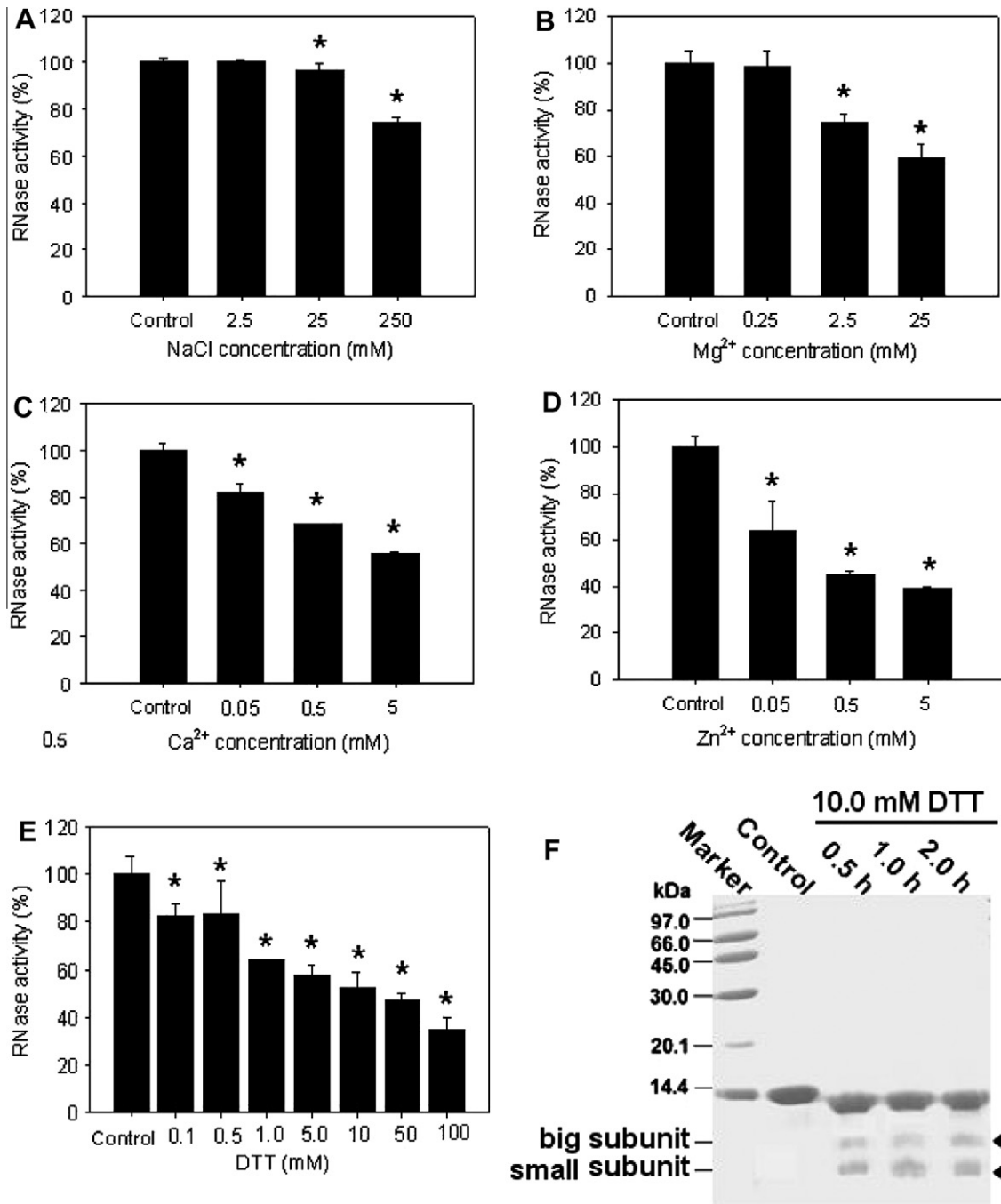


Fig. 6. Effect of cations and dithiothreitol (DTT) on RNase activity of pumpkin 2S albumin. (A–E) 200 μ g of RNA (type X) was incubated for 15 min at 37 °C with pumpkin 2S albumin (final concentration of 0.02 μ M) in a reaction volume of 150 μ l and with cations or DTT at the concentrations indicated. The RNase activity of each treatment was calculated as a percentage compared with negative control (in absence of cations or DTT). Means $\pm$ S.D. of three independent experiments are shown. (F) Effect of DTT treatment on the structure of pumpkin 2S albumin. Filled triangles indicated were the produced proteins of pumpkin 2S albumin big chain and small chain. Asterisks indicate statistically significant difference from control ($P < 0.05$).

3.3. RNase activity of pumpkin 2S albumin

The RNase activity of purified pumpkin 2S albumin was tested by using different tRNAs (Fig. 3A), polyhomoribonucleotides (Fig. 3B), and total RNAs (Fig. 4) as substrates. The values of its specific RNase activity were: 1926 U/mg toward type X tRNA, 9630 U/mg toward type III tRNA, and 5185 U/mg (toward type IV tRNA). It exhibited potent activity toward poly(U) (1481 U/mg), and moderate activity toward poly(A) (170 U/mg) and poly(C) (85 U/mg), but had no detectable effect on poly(G). Furthermore, when total RNA of either MCF-7 cells (Fig. 4A) or *B. variegata* seeds (Fig. 4B) was used as substrate, pumpkin 2S albumin caused significant digestion of all rRNAs (5S rRNA, 18S rRNA, and 28S rRNA) in a dose-dependent as well as time-dependent manner.

3.4. Biochemical characterization of its RNase activity

The temperature and pH dependence of the RNase activity of the pumpkin 2S albumin are shown in Fig. 5 which revealed that opti-

mum ambient temperature and pH for the RNase activity were 40 °C and from pH 7 to 8, respectively. The effects of different cations and DTT at various concentrations on RNase activity of pumpkin 2S albumin are shown in Fig. 6. NaCl manifested a meager suppression of the activity only when its concentration was augmented to 250 mM (Fig. 6A). Low doses of bivalent cations as Mg^{2+} (25 mM, Fig. 6B), Ca^{2+} (5 mM, Fig. 6C), and Zn^{2+} ions (5 mM, Fig. 6D) exhibited potent inhibition of the activity by 50–75%. Disruption of the disulfide bonds by DTT treatment brought about a loss of its RNase activity in a dose-dependent manner (Fig. 6E and F).

4. Discussion

A simple chromatographic procedure was applied for the purification of pumpkin 2S albumin with an acceptable yield. Sequence alignment and phylogenetic tree work unveiled a close relationship between pumpkin 2S albumin and pumpkin prepro2S albumin [13,14]. As reported by Hara-Nishimura et al. [13,14], a

EAL84471	1	--MLYKKII	AVAALLVAPVLAAPTEFDTRAC	-----	---DYTG	GSNCYSASAVRA
P00654	1	-----	CDIPQSTN	-----	---CGGNV	YSNDDINT
ACI86985	1	MKAFWRNAALLAVSL	LPSSANAVALQAKQY	GDFDRYVLALSWQTGF	---CQS	QHDRNRNERD
JC5560	1	-----	KETAAAKFERQHMD	SSSTAASSN	-----	YCNQMMKSRNLTKD
F84931	1	-----	MLNYFFKKQLRLSD	STSFQD	-----	VFNGSVKKKNTLEI
CAL98606.1	1	-----	MGYGGIVNKQQAEL	LNGIALAYVG	-----	DAIFEVFRDYLLKKG
EC	1	-----	AQVINTFDGVADYLL	TYHKLDPN	-----	YITKSEAQALGWV
This work	1	-----	RQGREERCROMSARE	EELRS	-----	CEQYLROQSRDVL
AAZ39212	1	MDKVEIYADGACKGN	PGTGGWGALMVADG	-----	---	AKKEIYGGELNTT
CAD44289	1	MAGPVKDREA	FQRLNFLYQAAHCVLAQDPEN	-----	---	QALARFYCYTERTIAK
consensus	1	.	.	.	.	.
EAL84471	46	AQEAGYELYSSDET	VGSNYPHKYNNYEGFD	-----	---FPVSS	---PYIEWPILSSGK
P00654	22	AIQALDDVANGDRP	--DNYPHQYYDEASED	-----	---ITLCCGSGPWSE	FPLVYNGP
ACI86985	61	ECRLQTETTNKADFL	TVHGLWPGLPKSVA	ARGV--	---DERRWMRFGC	ATRPINLPEARASR
JC5560	39	RCKP-VNTFVHESL	ADVQAVCSQKNVACKNG	-----	---QTNCYQSYSTMS	ITDCRE
F84931	35	SILGRFNLLGHPRLG	--LSIPRKNIKHAHNRN	-----	---LIKRLVRETFRLL	QKLLSMDF
CAL98606.1	41	LTKPAFLQKNATKFV	SAKAQAKIIRAMEEVD	FL--	---TEYELTYFKRGR	NAHSKTTAKNTDV
EC	37	ASKGNLADVAPGKS	IGGDI FSNREGKLP	AKSG--	-----	RTWREADINYTSG
This work	33	QMRGIENPWRREGG	SFDECCRELKNVDEECR	-----	---CDMLEEIIAREE	QRO
AAZ39212	43	NNRMELKAVIEALT	ALKRPCQVVLYTDSQ	YVQKGISEWIGHGW	KARGWKTAAKAPV	KNVDL
CAD44289	48	RLVLRDPSPVK	RTRLRCGCSLLVPGL	TCTQRRRCRGQRW	TVQTCLTCQRSQ	RFLNDPGH
consensus	61	..	.	.	.	.
EAL84471	94	IYSGG	-----	---SPGAD	-----	---RVV
P00654	71	YYSSRDN	-----	---YVSPGPD	-----	---RVI
ACI86985	119	MCSSPETGLSLETA	AKLSEVMPGAGGRS	CLERYEYAKHGAC	FGFDPDAYFGTM	VRNLNCEI
JC5560	87	TGSSK	-----	---YPNCA	-----	---YKTTQAN
F84931	87	VVIKKN	-----	---	---	---ILFL
CAL98606.1	99	VTYRISTG	-----	---FEAVVG	-----	---ILHLT
EC	82	FRNSD	-----	---	---	---RIL
This work	78	ARGQEGR	-----	---	---	---QML
AAZ39212	103	WQALDTA	-----	---	---	---QAV
CAD44289	108	LLWGDRPE	-----	---	---	---AQLG
consensus	121	.	.	.	.	...
EAL84471	107	FNSKD-ELAGL	-----	---ITHTGASGNN	---FVACT	-----
P00654	88	YQNTNGEFCAT	-----	---VTHTGAASYD	GGFTQCS	-----
ACI86985	179	KESEAGKFLADNYG	KTVSRRDPDAAF	AKSWGKENVKAVK	LTCQGNPAYL	TEIQVSIKADA
JC5560	104	KHIIV	-----	---ACEGN	FPYVPVHF	DASV
F84931	98	NNTR	-----	---IIDMLNSLWS	NYYR	-----
CAL98606.1	118	QENER	-----	---LKEFWN	FCVETIEKDTA	-----
EC	90	YSSDW	-----	---LIYKTTDHYK	TFTKIR	-----
This work	88	QKARN	-----	---	---	---LPSCGIRPQRCDF
AAZ39212	113	HEVEWRWVRGHN	-----	---GHPGNERADQ	LANLGVETVR	-----
CAD44289	120	SQADSKPLQP	-----	---LPNTAHSIS	DRLPPEKM	QTQG
consensus	181	..	.	.	.	.

Fig. 7. Sequence alignment between pumpkin 2S albumin and some representative endoribonucleases. GenBank accession numbers are: EAL84471(RntA), an extracellular guanylyl-specific RNase T1 from *Aspergillus fumigatus* Af293; P00654, an RNase U2 from *Ustilago sphaerogena*; ACI86985, an RNase I from *Escherichia coli*; JC5560, a pancreatic RNase A; F84931, an RNase P from *Buchnera* sp.; CAL98606, an RNase III from *Lactococcus lactis* subsp. *cremoris* MG1363; EC (EC3.1.27.1), an RNase T2 from *Bacillus circulans*; AAZ39212, an RNase H from *Janthinobacterium lividum*; CAD44289, an RNase V1 from *Homo sapiens*. The amino acid sequence of Q39649.1 (prepro2S albumin) is presented instead of the partial sequence of pumpkin 2S albumin in this work for sequence alignment.

signal peptide, a propeptide, a small chain, and a big chain constitute complete pumpkin prepro2S albumin (totally 141 amino acids, theoretical pI and MW were 5.74 and 16 596.82, respectively [15]). After cleaving the signal peptide, this prepro2S albumin is first converted into pro2S albumin (119 AA, pI/MW: 5.75/14321.04). And finally, with the assistance of a vacuolar processing enzyme to cleave the propeptide from the pro2S albumin, mature 2S albumin (106 AA, pI/MW: 7.70/12784.38) is produced. The pumpkin 2S albumin reported here appears to be the mature form of the pumpkin prepro2S albumin. Further work on full sequence determination is needed.

In order to determine the RNase activity of pumpkin 2S albumin, its purity was critical, inasmuch as the contamination of cellular proteins with nucleases is not uncommon [20]. The results of SDS-PAGE, MALDI-TOF mass spectrometry, and N-terminal sequencing confirmed that the acquired protein was highly purified (Fig. 1D and E). The specific RNase activity of pumpkin 2S albumin (1926 U/mg toward type X RNA) was comparable to that of the RNase from *Russula delica*, and higher than RNases from other mushrooms, including *Clitocybe maxima*, *Dictyophora indusiata*, and others [21]. Furthermore, its polyhomoribonucleotide specificity of poly(U) >> poly(A) > poly(C) > poly(G) was similar to some other RNases, including RNase from *Ganoderma lucidum* [22], RNase X₂ from the plant *Petunia inflata* and RNase T₂ from *Aspergillus oryzae* [10]. In addition, it manifested significant hydrolytic activity against total RNAs of MCF-7 cells and *B. variegata* seeds (Fig. 4).

The RNase activity exhibited a pH optimum of 7.0–8.0 and a temperature optimum of 40 °C which were the same as RNases of *P. inflata* [10]. The effects of different cations on its RNase activity were further investigated. Some RNases implement their RNA cleavage in a cation-dependent manner, such as the eukaryotic exosome needs Mg²⁺, Mn²⁺, and Zn²⁺ ions for its endonucleolytic activity [12], and Zc3h12a [5] and RNase BN [23] exhibit their endonuclease activities in a Mg²⁺-dependent manner. On the other hand, some cation-sensitive RNases have also been reported [10,11,20,24]. For instance, about 1 mM CuSO₄ or 1.0 mM ZnSO₄ could inhibit up to 75% of the RNase activity of the S-allele-associated protein of *P. inflata* [10]. The antagonistic effects of cations on the RNase activity of pumpkin 2S albumin were also noticed and the highest sensitivity was toward Zn²⁺ (Fig. 6). Potential mechanisms of this Zn²⁺-sensitive characteristic may be associated with its specific structure like the Zn²⁺-sensitive RNase AtCPSF30 [24]. The RNase activity of AtCPSF30 was inhibited by relatively high (>100 μM) concentrations of zinc, and this inhibition required a plant-specific N-terminal domain apart from the zinc finger core of the protein. Though low concentrations of zinc have no effect on their RNase activity since zinc and the zinc finger motifs are essential for the RNase activity, excessive zinc is toxic to the enzymes, producing changes including a change of structure and a reduction in cellular energy stores, among others [24–27]. The exact mechanism of inhibition of RNase activity of pumpkin 2S albumin by high concentrations of divalent metals needs to be further investigated.

Based on the results of sequence alignment and phylogenetic tree relationship with other endoribonucleases (Fig. 7) and exoribonucleases (Supplementary Fig. 3), and its relatively small molecular weight, pumpkin 2S albumin manifests a pretty close relationship with RNase H (accession number: AAZ39212.1), an endoribonuclease from *Janthinobacterium lividum*. Since the homology to other known RNases is not striking, pumpkin 2S albumin is probably a novel RNase. To know which amino acid residues are present in the catalytic cleft of a “typical” RNase for performing the catalytic action, further site-directed mutagenesis work in potential areas needs to be done. The molecular mechanism of its RNA cleaving activity may be analogous to the dynamic mechanism of RNA degradation by RNase II [4].

In sum, this work describes the purification and identification of a 2S albumin from pumpkin seeds. Its RNase activity was clearly elucidated. Whether other 2S albumins manifest RNase activity deserves further study. Since RNase activity has not been previously reported in 2S albumins, this work may shed additional light on biological significance of this group of proteins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.08.041.

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