

Expression, Purification, and Characterization of Almond (*Prunus dulcis*) Allergen Pru du 4

Yuzhu Zhang,^{*,†} Wen-Xian Du,[†] Cécile Fregevu,^{†,‡} Mahendra H. Kothary,[‡] Leslie Harden,[†] and Tara H. McHugh[†]

[†]U.S. Department of Agriculture, Agricultural Research Service, Pacific West Area, Western Regional Research Center, 800 Buchanan Street, Albany, California 94710, United States

[‡]U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 8301 Muirkirk Road, Laurel, Maryland 20708, United States

ABSTRACT: Biochemical characterizations of food allergens are required for understanding the allergenicity of food allergens. Such studies require a relatively large amount of highly purified allergens. The level of Pru du 4 in almond is low, and its expression in a soluble form in *Escherichia coli* required an expression tag. An MBP tag was used to enhance its expression and solubility. Sumo was used for the first time as a peptidase recognition site. The expression tag was removed with a sumo protease, and the resulting wild-type Pru du 4 was purified chromatographically. The stability of the allergen was investigated with chemical denaturation. The Gibbs free energy of Pru du 4 folding–unfolding transition was determined to be 5.4 ± 0.7 kcal/mol.

KEYWORDS: food allergy, sumo, tree nut allergy, stability, almond, allergen

■ INTRODUCTION

Most food allergic reactions are initiated by the immunoglobulin E (IgE) mediated recognition of allergenic proteins.^{1–3} Food allergy has become a major problem in the developed countries.^{4–6} Extensive research has been devoted to allergy research in the past two decades,^{4,7} but most of the efforts are directed toward the understanding of the pathogenesis and mechanisms of the responses of the human immune system to the triggers of allergic reactions. Despite the progresses being made in understanding the immune responses in an allergic reaction, the current management of food allergies stresses strict avoidance of the allergens due to the lack of a cure.⁸

Food proteins that cause allergic reactions belong to only a few protein families, including the profilin family. Ubiquitously present in all eukaryotic cells, profilins regulate various cellular processes in mammalian as well as in plant cells.^{9,10} Profilin is referred to as a panallergen because it has been known to be pollen allergens and/or food allergens in numerous species.^{11,12} Highly purified profilin allergens from more than one food are needed to understand the allergenicity of this food protein and its role in allergen cross-reactivity. Albeit ubiquitous, the amount of profilin is minute, making it desirable to express and purify profilins using recombinant technology.

A number of expression tags are used widely to enhance the exogenous expression of desired proteins in *Escherichia coli*, including GST, MBP, TXN, sumo, etc.,^{13–17} including a recent study that reported sumo-tagged expression of *Schistosoma japonicum* profilin.¹⁸ A peptidase recognition site is usually engineered between the fusion tag and the target protein. A number of peptidases are widely used for removing the fusion tags.¹⁴ The specificities of the peptidases vary, and careful optimization is required to achieve maximum yield of the target protein. Most of the widely used peptidases leave extra amino acids in the N-terminal of the target protein after the removal of the fusion tag. We are interested in studying the relative

importance of linear and conformational IgE epitopes in food allergens. Native allergens without any extra amino acids left over in their N-terminal are required for research on IgE epitopes. Here we report the results of the expression of Pru du 4 with sumo as a peptidase recognition site in the fusion protein, the purification of the native almond allergen Pru du 4, and the investigation of the stability of this food allergen.

■ MATERIALS AND METHODS

Plasmid Construction and Protein Expression. Young Non-pareil almond was collected from a local orchard with the help of the Almond Board of California. mRNA was prepared from almond kernels with the RNeasy Plant Mini Kit (QIAGEN, Valencia, CA) following the manufacturer's protocols. Briefly, 100 mg of the watery kernel of an almond were placed in a mortar containing enough liquid nitrogen to keep the sample submerged and ground with a pestle. After grinding, the powder and the liquid nitrogen were transferred to an RNase-free, DNase-free, and liquid-nitrogen-cooled microcentrifuge tube (2 mL). As soon as the liquid nitrogen was evaporated, 450 μ L of Buffer RLT was added to the sample. The sample was then vigorously vortexed, and the amount of the sample corresponding to 50 mg of kernel was transferred to a QIAshredder spin column and centrifuged for 2 min at 13 000 rpm in a microcentrifuge. The supernatant of the filtrate was transferred to an RNAeasy spin column placed in a 2 mL collection tube and spun for 2 min at 13 000 rpm. Washing of the column and RNA elution were carried out according to the kit protocol including the usage of the optional steps. Fifty milliliters of Milli-Q water was used to elute the RNA.

An Omniscript Reverse Transcription Kit (QIAGEN) was used to synthesize cDNAs from the total RNA. The RT reaction was carried out according to the manufacture's protocols using 5 μ M of a 20-nucleotide poly dT primer and 2 μ L of the fresh total RNA as template

Received: September 17, 2014

Revised: December 1, 2014

Accepted: December 1, 2014

Published: December 1, 2014

in a 20 μ L RT mixture. The RT reaction was allowed to proceed for 60 min at 37 °C in the presence of a recombinant ribonuclease inhibitor (RNaseOUT from Invitrogen) at a concentration of 2 units/ μ L. The reverse transcriptase was inactivated by incubating the reaction mixture at 95 °C for 5 min. The RT product was either used immediately as a template in PCR or stored at –80 °C for future use.

Primers I (5'-caccgtgaacagatcggtgatgtctggcagcagctacgtc-3') and II (5'-cctctcgagttacagaccctgctcgataaggtta-3') were designed based on a published Pru du 4 sequence (GI:24473793)¹⁹ to amplify the coding region of Pru du 4. The underlined portion of primer I was added to facilitate a second round PCR to join the Pru du 4 coding sequence with that of the sumo. The sumo sequence was previously synthesized commercially with codon optimization for *E. coli* expression. Primer III (5'-ccggtactctgggaagttaaacggaaaccc-3') and primer IV (which is complementary to primer I) were used to amplify the synthesized sumo coding sequence. The two PCR products were mixed and used as template for a second round PCR using primers III and II. The final PCR product was inserted into the cloning vector pBluescript II SK with blunt-end ligation to make pBlue-SPru4. The ligation product was transformed to *E. coli* strain DH5 α , and the plasmid prepared from the isolated colonies was sequenced to confirm correct insertion of the sumo-Pru du 4 fusion. pBlue-SPru4 was digested with *Bam*HI and *Xho*I to release the sumo-Pru du 4 coding sequence which was then ligated with the pRSFdeut-1 vector prepared with the same enzymes to generate pRSF-SPru4.

Primers V (5'-cctcatgagccagctgcatcaccatcatcaccagtcctttaaatacgaagaggttaac tggtaac-3') and VI (5'-ccagatcttacgtaagtctgcgcgg-3') were used in a PCR with pMAL-c2 as template to amplify the MBP coding sequence. The underlined sequences in the primers were added to incorporate enzyme recognition sites and a His-tag coding sequence. The PCR product was inserted into Bluescript II SK as described above. The MBP coding sequence was confirmed with DNA sequencing. It was released with *Bsp*HI and *Bgl*II and ligated with the pRSF-SPru4 plasmid prepared with *Nco*I and *Bam*HI to generate pRSF1MS-SPru4. The sequence-confirmed pRSF1MS-SPru4 plasmid was then transformed into the *E. coli* strain BL21(DE3)* (Invitrogen) for protein expression. The transformed bacteria were grown in 1 L batches of LB medium containing 50 mg/L kanamycin (Gold Biotechnology, St. Louis, MO) at 37 °C until the OD₆₀₀ of the culture reached 1.0. IPTG (Gold Biotechnology) was then added to the medium to a final concentration of 1 mM, and the expression of Pru du 4 fusion protein was allowed for 5 h. The cells were collected by centrifugation, and the cell pellets were stored at –80 °C for future use.

Purification of the Fusion Protein. The cell pellet was thawed on ice and resuspended in 50 mL of a His-binding buffer (buffer HB, 20 mM imidazole, pH 8.0, 500 mM NaCl) containing 1 mM benzamidine. The bacteria were lysed by sonication for 9 min in a beaker surrounded by a mixture of ice and water using a Model 450 Digital Sonifier (Branson, Danbury, CT). The cell lysate was centrifuged at 20 000g for 40 min at 277 K. The supernatant was loaded onto a 5 mL HisTrap FF Crude column (GE Healthcare, Piscataway, NJ). The column was washed with 7 bed volumes of buffer HB and then eluted with a His-elution buffer (buffer HE, 300 mM imidazole, pH 8.0).

The main peak of the HisTrap elution was diluted with buffer QB (10 mM Tris-HCl, pH 7.9) to 50 mL. The sample was then loaded onto an 8 mL Source15 Q column (GE Healthcare). The column was washed with 7 bed volumes of buffer QB and eluted with QB plus a linear NaCl gradient of 0–1 M (over 10 bed volumes). The main peak of the elution was confirmed later to contain the target fusion protein.

Removal of the Fusion Tag. The elution fractions of the main peak from the anion exchange column were pooled, and aliquots of the pooled sample were used to optimize the conditions for the removal of the fusion tag by a recombinant sumo protease rULP which also contained a His-tag and was expressed and purified in-house. The scaled up rULP digestion was in 50 mL of a buffer containing 10 mM Tris, pH 7.9, 250 mM NaCl, 18 mg of His-MBP-sumo-Pru du 4, and 18 μ g of rULP. The digestion was carried out at 20 °C for 12 h. The digested sample was passed through a 5 mL HisTrap FF Crude

column, and the flow-through was collected and concentrated to a final volume of 15 mL using an Amicon Ultra centrifugal filter device with 3 kDa molecular weight cutoff for proteins (Millipore, Bedford, MA). The concentrated sample was further purified with size exclusion chromatography (SEC) using an XK 26/70 Superdex-75 column (GE Healthcare) pre-equilibrated with buffer SC (10 mM Tris-HCl, pH 7.9, 200 mM NaCl) and eluted with the same buffer. Determination of the concentrations of the purified protein samples was based on OD₂₈₀ using the theoretical molecular mass and molar extinction coefficient using the program SPHERE (<http://www.fccc.edu/research/labs/roder/sphere/>).²⁰ All chromatographic steps were carried out at room temperature using an FPLC system (GE Healthcare).

Samples of the target protein after each step of purification were boiled in an SDS sample buffer for 5 min and separated by electrophoresis with 4–20% gels and stained using the Colloidal Coomassie G-250 Staining protocols.²¹ The image of the stained gel was taken with a Nikon digital camera.

N-terminal Sequencing. The final recombinant Pru du 4 was subjected to SDS-PAGE using 8–25% gradient gels in a PhastSystem (GE Healthcare) and electrophoretically transferred (0.35 A at 25 °C) to a Problott membrane (Applied Biosystems, Foster City, CA) in a transfer buffer [10 mM 3-(cyclohexyl-amino)-1-propanesulfonic acid containing 10% methanol, pH 11.0]. The blot was stained with Coomassie Brilliant Blue, and the protein band was excised and subjected to N-terminal amino acid sequencing by Edman degradation using a Shimadzu PPSQ-33A Protein Sequencer (Shimadzu, Columbia, MD).

Determination of the Mass of the Purified Protein. The purified Pru du 4 was subjected to reverse-phase HPLC using an Easy nLC II (Thermo Fisher Scientific, San Jose, CA) with a PicoChip column packed with 3 μ m Reprosil-Pur (New Objectives, Woburn, MA) and a flow rate of 250 nL/min. MS data were collected on an Orbitrap Elite operated at 15 000 mass resolution. Data across chromatographic peak were averaged with Thermo Excalibur software and subsequently deconvoluted with MagTran software.²²

Circular Dichroism (CD). The buffer of the purified protein was changed to a buffer containing 20 mM phosphate (pH 7.0) and 200 mM NaCl by repeated concentration-and-dilution using an Amicon Ultra centrifugal filter device with 3 kDa molecular weight cutoff for proteins (Millipore). CD spectroscopy was used to study the secondary structure content of recombinant Pru du 4. Far-UV spectra were collected in the 195–260 nm region using a J-815 CD spectrometer (Jasco, Tokyo) and a quartz cuvette with a light path length of 2.0 mm. Wavelength scans were performed with a speed of 50 nm/min at 20 °C. The spectra were averaged for 10 scans.

Guanidinium Hydrochloride (GuHCl) Denaturation. Two stock solutions of recombinant Pru du 4 were prepared. Both solutions (pH 7.0) contained 2 μ M of the protein, 10 mM of phosphate, and 200 mM of NaCl. One of them additionally contained 6.923 M of GuHCl. Thirty samples were prepared with various ratios of the two stock solutions to obtain samples at 0.187 M intervals of GuHCl concentration from 0 to 5.43 M. A 6 M GuHCl sample was also made by mixing the two stock solutions at the right ratio. The samples were thoroughly mixed and equilibrated at room temperature for 24 h. The CD signals at 220 nm of these samples were measured at 20 °C for half a minute with data collected every second. The free energy of denaturation was calculated by fitting the data to a two-state transition using Origin Pro9 software (OriginLab Corp., Northampton, MA).

RESULTS

Isolation and Expression of Pru du 4. The mRNA sequence of Pru du 4 could be found in the Genbank that also contained the 5'- and 3'-untranslated regions (GI:24473793). On the protein level, it has 100% sequence identity with the Japanese apricot profilin (GI:645226637) and 99% (GI:596206424)–100% (GI:27528309) to the peach profilin allergen Pru p 4.²³ Using primers designed based on the available Pru du 4 mRNA sequence, the coding sequence for

the Nonpareil almond profilin isolated in this study was identical to that of the Pru du 4 in the GenBank. When Pru du 4 was expressed without an expression tag in *E. coli*, only minimal expression could be achieved. This is also true when expressed with a sumo tag (data not shown). We sought to use sumo as an enzyme recognition site and produce Pru du 4 with a protocol depicted in Figure 1. Despite of its size, using sumo

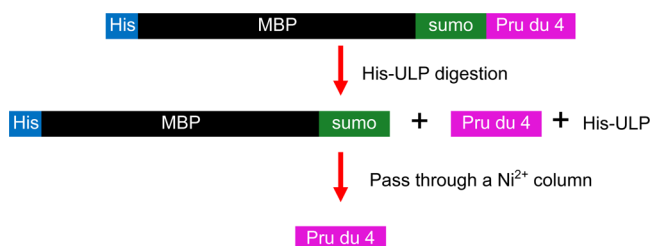


Figure 1. Producing Pru du 4 with sumo as a peptidase recognition site.

as a peptidase recognition site had no negative effect on the enhancement of the expression of Pru du 4 by MBP fusion (Figure 2, lane 1, and data not shown). Sumo can be used as a peptidase recognition site at least in the combination of MBP-sumo and Pru du 4.

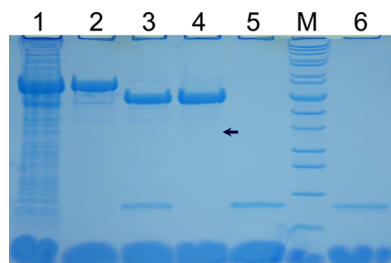


Figure 2. SDS-PAGE analyses of recombinant Pru du 4. Samples loaded in lanes 1–6 are (1) supernatant of the lysate of *E. coli* expressing Pru du 4, (2) sample after ion exchange purification, (3) product of ULP digestion, (4) unwanted proteins removed from the protease digestion product, (5) recombinant Pru du 4 after tag-removal, (6) the final Pru du 4 sample after SEC. The molecular masses (in kDa) of the protein standard in the maker (lane M) are 200, 150, 120, 100, 85, 70, 60, 50, 40, 30, 25, 20, 15, and 10, respectively. The arrow line close to lane 4 indicated the position of the ULP band that is barely detectable in lanes 3 and 4.

Purification of Pru du 4. The expressed His-MBP-sumo-Pru du 4 was highly soluble in aqueous solutions. The fusion protein was purified with immobilized metal affinity chromatography using a Ni^{2+} column. The purity of the sample was >90%, high enough to start fusion tag removal. To facilitate downstream purification, a second step of purification (anion exchange chromatography) was performed. In the ion exchange step, the sample eluted at ~200 mM NaCl. This step also eliminated imidazole from the sample. High concentration of imidazole is undesirable for removing the expression tag from the sample after protease digestion. Recombinant ULP produced in our lab was used to cleave the fusion tag. After optimization, a peptidase/substrate ratio of (w/w) 1:1000 was used. The reaction was carried out at 20 °C overnight, and it was almost complete without nonspecific digestions (Figure 2, lane 3). The undigested fusion protein, the cleaved fusion tag, and the recombinant ULP were successfully removed by

passing the digestion product through a Ni^{2+} column (Figure 2, lane 4). The flow-through seemed to contain homogeneous recombinant Pru du 4 (Figure 2, lane 5). Gel filtration was used to further purify the recombinant Pru du 4 to ensure high purity. The yield for the final product was at least 8 mg/L. Twenty cycles of N-terminal amino acid sequencing of the sample confirmed the expected N-terminal sequence (MSWQQYVDDHLMCDIDGNRL) of Pru du 4, except for Cys13 for which no major signal was obtained as is the case usually for a Cys residue. In addition, the total mass of the protein was determined to be 14060.8 Da (Figure 3). This is consistent with the calculated isotope averaged mass of 14061.10 for the protein (using the ExPASy server at http://web.expasy.org/cgi-bin/compute_pi/pi_tool). Additionally, the mass spectrometry method also detected minor populations of the protein with Pyruvoyl-(Serine) or oxidized residues that are most likely at its methionine residues.

Chemical Stability of Pru du 4. Far-UV CD spectrometry studies indicated that the spectrum of recombinant Pru du 4 is typical of a protein with mixed α -helix and β -sheet secondary structures (Figure 4). Chemical denaturation is widely used as a way to assess the stability of native proteins. The folding–unfolding equilibrium of Pru du 4 was studied by measuring the GuHCl-induced transitions of the protein. In the present study, the equilibrium between unfolded and folded Pru du 4 at various concentrations of GuHCl was studied by measuring the CD signal at 220 nm (Figure 5). The data points seemed to follow a sigmoidal shape, indicative of a two-state transition between folded and unfolded states of Pru du 4 without a measurably populated stable intermediate. The method for finding the free energy of unfolding of a protein has been described in many comprehensive reviews (see, e.g., ref 24). The concentrations of the protein in the unfolded [U] and folded [F] states vary with the change of the denaturant concentration. The Gibbs free energy of the equilibrium transition, $\Delta G = \ln([U]/[F])$, changes accordingly. To the first approximation, the free energy of transition is a line function of the denaturant concentration

$$\Delta G = \Delta G_0 - mC$$

where C is the concentration of the denaturant, ΔG_0 is the free energy of the transition when there is no denaturant, and m is a constant. At the midpoint of the transition (when $C = C_m$), the folded and unfolded states are equally populated and $\Delta G = 0$. Thus, m equals to $\Delta G_0/C_m$. Generally, the CD signals of a protein in its folded and unfolded are both a linear function of the concentration of the denaturant. The measured CD signal is the sum of those of the folded and unfolded protein. Thus, ΔG_0 , m , and C_m can be found by nonlinear fitting of the CD data. The results of the curve fitting are shown in Table 1. The transitions occurred with a midpoint concentrations of 1.38 ± 0.02 M. The free energy of unfolding, ΔG_0 , in the absence of denaturant was 5.4 ± 0.6 kcal/mol.

DISCUSSION

Profilin is considered to be a panallergen. Almond profilin is known to be a food allergen. Unlike the 2S albumin, 7S vicilin, and 11S legumin allergens, the amount of profilin, including almond allergen Pru du 4, in plant seeds is minute. Expression of Pru du 4 in *E. coli* without an expression tag was unsuccessful though untagged expression of peanut profilin Ara h 5 was successful.²⁵ Its expression with an MBP-tag was reported, and the recombinant protein was used in immunological analyses.¹⁹

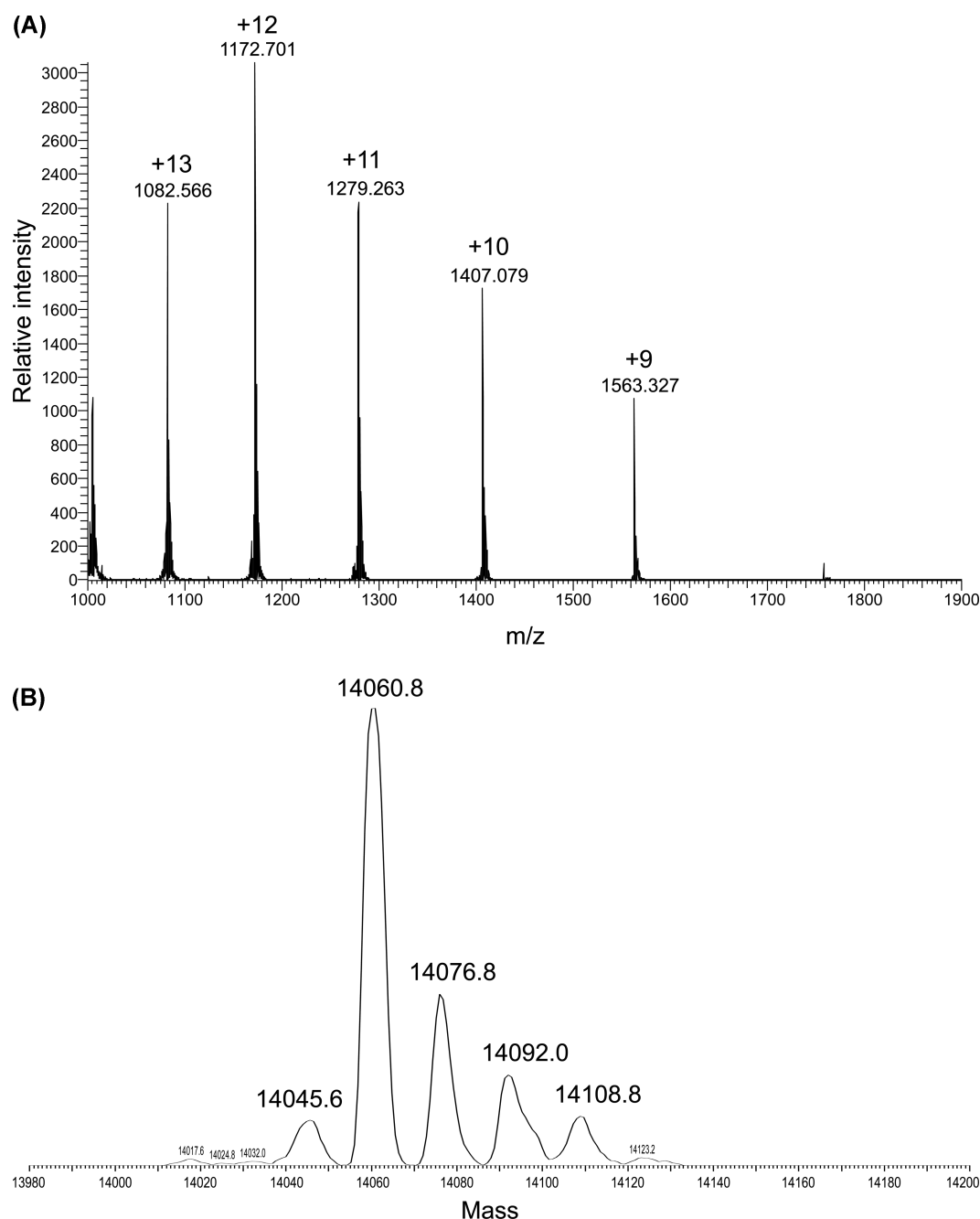


Figure 3. Mass determination of the purified Pru du 4. The sample was eluted from a Reprosil-Pur column coupled with an Easy nLC II mass spectrometer for accurate mass measurement. Panel A shows the mass spectrum of purified Pru du 4. Panel B shows the deconvolution result of the spectrum. The main peak indicated the measured mass of the protein is within 0.3 mass units of its calculated mass based on its peptide sequence.

In dot blot and ELISA studies, the recombinant protein and Pru du 4 purified from almond extract were recognized by IgE in patient sera differently. Some of the patient sera recognize Pru du 4 from almond, but not the recombinant Pru du 4.¹⁹ N-terminal amino acid sequencing of Pru du 4 purified from almond indicated that no signal peptide was removed post-translationally (ref 19 and above). One of the reasons for the differences in the IgE recognition of the two versions of the allergen might be that the recombinant Pru du 4 used in the reported study had eight extra amino acids at its N-terminus after removing the expression tag with thrombin (or factor Xa) cleavage. These residues might block the conformational epitopes for IgE association.

To understand the relative importance of linear and conformational IgE epitopes in food allergy, native allergens are needed. As the amounts of a number of the allergens in foods are very low, it is desirable to obtain the native allergen with recombinant technology. In addition, an available system for producing native allergen in *E. coli* will make it possible to study the allergenicity of food allergens with site-directed mutagenesis technologies. Thus, we decided to use a highly specific peptidase for removing the expression tag without leaving any extra amino acids in the final product. Most of the widely used protease recognition sites that have been incorporated in various DNA vectors designed for protein expression are short peptides with just a few amino acids. To

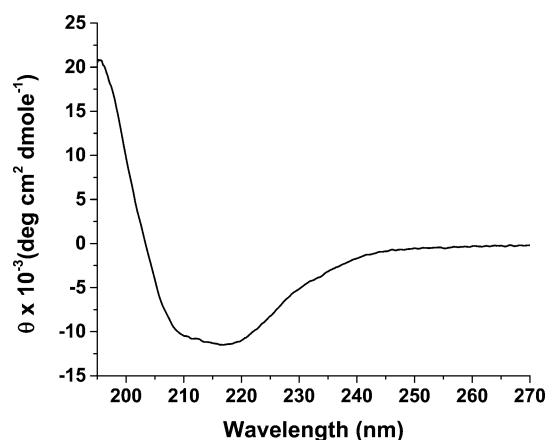


Figure 4. CD spectrum of recombinant Pru du 4. The residue molar ellipticity is shown as a function of wavelength.

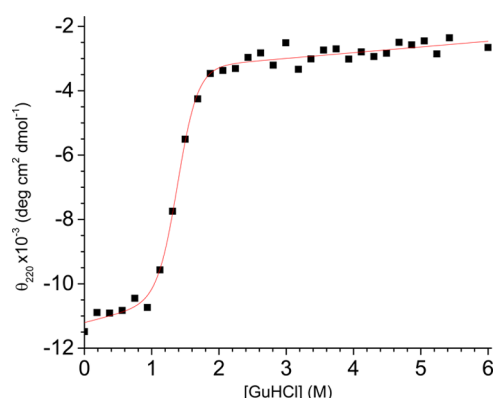


Figure 5. Folding–unfolding equilibrium of Pru du 4 in the presence of GuHCl. The data were fitted according to the equations shown in the text.

Table 1. Equilibrium Parameters for GuHCl-Induced Unfolding of Pru du 4^a

ΔG_0 (kcal mol ⁻¹)	C_m (M)	m (kcal mol ⁻¹ M ⁻¹)	R^2
5.4 ± 0.7	1.38 ± 0.02	3.9 ± 0.5	0.996

^aMeasured by CD at 220 nm in 10 mM sodium phosphate, pH 7.00, 20 °C.

our knowledge, the use of a protein, such as sumo, as a protease recognition site has not been reported, other than when sumo itself was used as an expression/solubility enhancement tag. Our results showed that by using sumo for peptidase recognition, the tag could be removed from almost 100% of the fusion protein without nonspecific cleavage of the target protein or the fusion tag. In our experience, this is a better system than using other proteases such as enteropeptidase which also does not leave any extra residues to the target protein. We expect that sumo as a protease recognition site can be used to conveniently obtain a wide variety of proteins. This may become the recognition site of choice especially when extra residues at the N-terminal of the target protein become problematic.

Profilin is considered as a panallergen.^{11,12} Celery allergen Api g 4, which is also a profilin, was reported to lose its association with patient serum IgE after it was heated at 100 °C.²⁶ Strong allergenicity of cherry allergens was reported to be related to high stability against thermal processing and

digestion.²⁷ The ΔG_0 value obtained for Pru du 4 was 5.4 ± 0.7 kcal/mol. This is similar to the free energy of the folding–unfolding transitions of other globular proteins in the ProTherm Database.²⁸ The database currently consists of more than 3000 entries of the thermodynamic parameters for wild-type proteins obtained by studying the chemically induced changes in the folding–unfolding equilibrium of the proteins. While the stability is considered a contribution factor for the allergenicity of food proteins, information on the stabilities of food allergens derived from careful biochemical studies is limited. Additional comparative studies of the thermodynamic parameters of food allergens are required to understand the relationship between the chemical, physical, and biological stability of food allergens and their allergenicity. Currently, a well-characterized IgE or IgG antibody that recognizes only the native Pru du 4 is not available although immunoblotting experiments with such antibodies might give additional indication on the immunological equivalence of the recombinant protein and the protein purified from almond. Thus, the development of well-characterized antibodies is also desired for better understanding of the allergenicity of food proteins.

AUTHOR INFORMATION

Corresponding Author

*Tel.: 510 559 5981. Fax: 510 559 5818. E-mail: yuzhu.zhang@ars.usda.gov.

Present Address

[†]Ecole d'Ingenieurs de Purpan, 75, voie du TOEC-BP 57611, 31076 Toulouse, CEDEX 3, France.

Notes

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

The authors declare no competing financial interest.

REFERENCES

- (1) Nowak-Wegrzyn, A. Food allergy to proteins. *Nestle Nutr. Workshop Ser. Pediatr. Program* **2007**, *59*, 17–31 discussion 31–6.
- (2) Asero, R.; Ballmer-Weber, B. K.; Beyer, K.; Conti, A.; Dubakiene, R.; Fernandez-Rivas, M.; Hoffmann-Sommergruber, K.; Lidholm, J.; Mustakov, T.; Oude Elberink, J. N.; Pumphrey, R. S.; Stahl Skov, P.; van Ree, R.; Vlieg-Boerstra, B. J.; Hiller, R.; Hourihane, J. O.; Kowalski, M.; Papadopoulos, N. G.; Wal, J. M.; Mills, E. N.; Vieths, S. IgE-mediated food allergy diagnosis: Current status and new perspectives. *Mol. Nutr. Food Res.* **2007**, *51*, 135–47.
- (3) Ozol, D.; Mete, E. Asthma and food allergy. *Curr. Opin. Pulm. Med.* **2008**, *14*, 9–12.
- (4) Sampson, H. A. Update on food allergy. *J. Allergy Clin. Immunol.* **2004**, *113*, 805–19 quiz 820.
- (5) Wang, J.; Sampson, H. A. Food anaphylaxis. *Clin. Exp. Allergy* **2007**, *37*, 651–60.
- (6) Branum, A. M.; Lukacs, S. L. Food allergy among U.S. children: Trends in prevalence and hospitalizations; NCHS Data Brief No. 10; National Center for Health Statistics: Hyattsville, MD, 2008.
- (7) Otsu, K.; Dreskin, S. C. Peanut allergy: An evolving clinical challenge. *Discov. Med.* **2011**, *12*, 319–28.
- (8) Anagnostou, K.; Clark, A. The management of peanut allergy. *Arch. Dis. Child.* **2014**.
- (9) Birbach, A. Profilin, a multi-modal regulator of neuronal plasticity. *Bioessays* **2008**, *30*, 994–1002.
- (10) Kandasamy, M. K.; Burgos-Rivera, B.; McKinney, E. C.; Ruzicka, D. R.; Meagher, R. B. Class-specific interaction of profilin and ADF isoforms with actin in the regulation of plant development. *Plant. Cell* **2007**, *19*, 3111–26.

- (11) Scala, E.; Alessandri, C.; Palazzo, P.; Pomponi, D.; Liso, M.; Bernardi, M. L.; Ferrara, R.; Zennaro, D.; Santoro, M.; Rasi, C.; Mari, A. IgE recognition patterns of profilin, PR-10, and tropomyosin panallergens tested in 3,113 allergic patients by allergen microarray-based technology. *PLoS One* **2011**, *6*, e24912.
- (12) Hauser, M.; Roulias, A.; Ferreira, F.; Egger, M. Panallergens and their impact on the allergic patient. *Allergy Asthma Clin. Immunol.* **2010**, *6*, 1.
- (13) Walls, D.; Loughran, S. T. Tagging recombinant proteins to enhance solubility and aid purification. *Methods Mol. Biol.* **2011**, *681*, 151–75.
- (14) Esposito, D.; Chatterjee, D. K. Enhancement of soluble protein expression through the use of fusion tags. *Curr. Opin. Biotechnol.* **2006**, *17*, 353–8.
- (15) Marblestone, J. G.; Edavettal, S. C.; Lim, Y.; Lim, P.; Zuo, X.; Butt, T. R. Comparison of SUMO fusion technology with traditional gene fusion systems: Enhanced expression and solubility with SUMO. *Protein Sci.* **2006**, *15*, 182–9.
- (16) Guo, W.; Cao, L.; Jia, Z.; Wu, G.; Li, T.; Lu, F.; Lu, Z. High level soluble production of functional ribonuclease inhibitor in *Escherichia coli* by fusing it to soluble partners. *Protein Expr. Purif.* **2011**, *77*, 185–92.
- (17) Huang, J.; Cao, L.; Guo, W.; Yuan, R.; Jia, Z.; Huang, K. Enhanced soluble expression of recombinant *Flavobacterium heparinum* heparinase I in *Escherichia coli* by fusing it with various soluble partners. *Protein Expr. Purif.* **2012**, *83*, 169–76.
- (18) Vervaet, N.; Kallio, J. P.; Meier, S.; Salmivaara, E.; Eberhardt, M.; Zhang, S.; Sun, X.; Wu, Z.; Kursula, P.; Kursula, I. Recombinant production, crystallization and preliminary structural characterization of *Schistosoma japonicum* profilin. *Acta Crystallogr.* **2013**, *F69*, 1264–7.
- (19) Tawde, P.; Venkatesh, Y. P.; Wang, F.; Teuber, S. S.; Sathe, S. K.; Roux, K. H. Cloning and characterization of profilin (Pru du 4), a cross-reactive almond (*Prunus dulcis*) allergen. *J. Allergy Clin. Immunol.* **2006**, *118*, 915–22.
- (20) Zhang, Y.-Z. *Protein and peptide structure and interactions studied by hydrogen exchange and NMR*. PhD Thesis, University of Pennsylvania, 1995.
- (21) Dybala, N.; Metzger, S. Fast and sensitive colloidal coomassie G-250 staining for proteins in polyacrylamide gels. *J. Vis. Exp.* **2009**, 1431.
- (22) Zhang, Z.; Marshall, A. G. A universal algorithm for fast and automated charge state deconvolution of electrospray mass-to-charge ratio spectra. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 225–33.
- (23) Rodriguez-Perez, R.; Fernandez-Rivas, M.; Gonzalez-Mancebo, E.; Sanchez-Monge, R.; Diaz-Perales, A.; Salcedo, G. Peach profilin: Cloning, heterologous expression and cross-reactivity with Bet v 2. *Allergy* **2003**, *58*, 635–40.
- (24) Pace, C. N. Determination and analysis of urea and guanidine hydrochloride denaturation curves. *Methods Enzymol.* **1986**, *131*, 266–80.
- (25) Wang, Y.; Fu, T. J.; Howard, A.; Kothary, M. H.; McHugh, T. H.; Zhang, Y. Crystal structure of peanut (*Arachis hypogaea*) allergen Ara h 5. *J. Agric. Food Chem.* **2013**, *61*, 1573–8.
- (26) Jankiewicz, A.; Baltes, W.; Bogl, K. W.; Dehne, L. I.; Jamin, A.; Hoffmann, A.; Haustein, D.; Vieths, S. Influence of food processing on the immunochemical stability of celery allergens. *J. Sci. Food Agric.* **1997**, *75*, 359–370.
- (27) Scheurer, S.; Lauer, I.; Foetisch, K.; San Miguel Moncin, M.; Retzek, M.; Hartz, C.; Enrique, E.; Lidholm, J.; Cistero-Bahima, A.; Vieths, S. Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. *J. Allergy Clin. Immunol.* **2004**, *114*, 900–7.
- (28) Kumar, M. D.; Bava, K. A.; Gromiha, M. M.; Prabakaran, P.; Kitajima, K.; Uedaira, H.; Sarai, A. ProTherm and ProNIT: Thermodynamic databases for proteins and protein–nucleic acid interactions. *Nucleic Acids Res.* **2006**, *34*, D204–6.